P2 6118

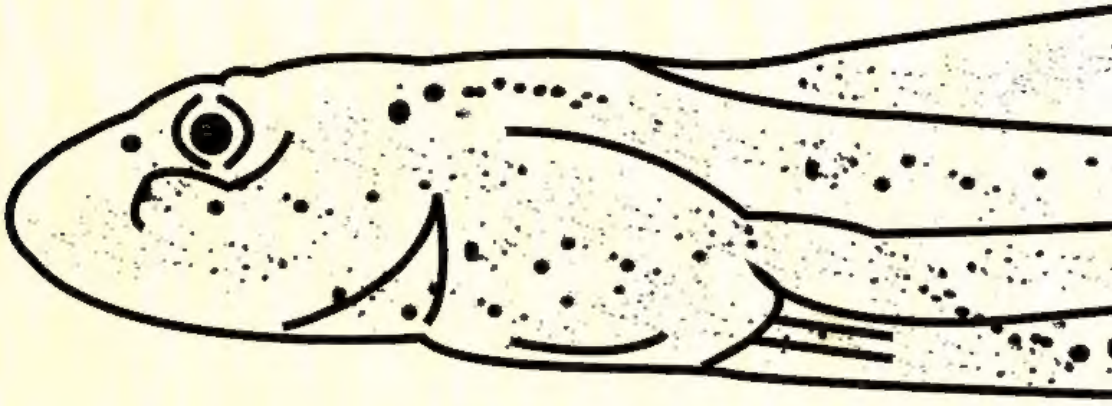
ISSN 0753-4973

ALYTES

INTERNATIONAL JOURNAL OF BATRACHOLOGY

The Amphibian Research and Monitoring Initiative

Proceedings of a Symposium held in Norman, Oklahoma, USA, 2004



Edited by C. Kenneth DODD, Jr

02 JUIN 2005

May 2005 **Volume 22, N° 3-4**

Source : MNHN, Paris

ISSCA

International Society for the Study and Conservation of Amphibians

(International Society of Batrachology)

SEAT

Laboratoire des Reptiles et Amphibiens, Muséum national d'Histoire naturelle, 25 rue Cuvier, 75005 Paris, France. – Tel.: (33).(0)1.40.79.34.87. – Fax: (33).(0)1.40.79.34.88. – E-mail: dubois@mnhn.fr.

BOARD

President: C. Kenneth DODD, Jr. (Gainesville, USA).
General Secretary: Annemarie OHLER (Paris, France).
Deputy Secretary: Alain PAGANO (Angers, France).
Treasurer: Stéphane GROSJEAN (Paris, France).
Deputy Treasurer: Rafael DE SÁ (Richmond, USA).
Councillors: Lauren E. BROWN (Normal, USA); Alain DUBOIS (Paris, France); JIANG Jianping (Chengdu, China); Esteban O. LAVILLA (San Miguel de Tucumán, Argentina); Thierry LODÉ (Angers, France); Miguel VENCES (Amsterdam, The Netherlands).

TARIFF FOR 2004

INDIVIDUALS

Regular 2004 subscription to *Alytes* (volume **22**) + ISSCA + *Circalytes*	50 € or $
Student 2004 subscription to *Alytes* (volume **22**) + ISSCA + *Circalytes*	25 € or $
SPECIAL OFFER: gift half-price subscription of one year for a colleague of your choice:	**Half of price above**
Regular 2004 subscription to *Alytes* (volume **22**) alone	46 € or $
Student 2004 subscription to *Alytes* (volume **22**) alone	23 € or $
Back issues of *Alytes*: any single issue	15 € or $
Back issues of *Alytes*: any double issue	25 € or $
Back issues of *Alytes*: any complete volume (4 issues)	40 € or $
Back issues of *Alytes*: complete set of volumes **1** to **21** (1982-2004)	630 € or $
Regular five-year (2004-2008, volumes **22** to **26**) individual subscription to *Alytes* + ISSCA + *Circalytes*	225 € or $
Regular five-year (2004-2008, volumes **22** to **26**) individual subscription to *Alytes*	200 € or $
SPECIAL OFFER: five-year (2004-2008, volumes **22** to **26**) individual subscription to *Alytes* + ISSCA + *Circalytes*, with complete set of back issues of *Alytes* (1982-2004, volumes **1** to **21**)	725 € or $
Life individual subscription to *Alytes* from 2004 on	1080 € or $
Life individual subscription to *Alytes* + ISSCA + *Circalytes* from 2004 on	1200 € or $
Patron individual subscription to *Alytes* from 2004 on	2160 € or $ **or more**
Patron individual subscription to *Alytes* + ISSCA + *Circalytes* from 2004 on	2400 € or $ **or more**

Important notice: from 1996 on, any new life or patron individual subscriber to *Alytes* is offered a free complete collection of back issues of *Alytes* from the first issue (February 1982) until the start of her/his subscription.

INSTITUTIONS

2004 subscription to *Alytes* (volume **22**) + ISSCA + *Circalytes*	100 € or $
2004 subscription to *Alytes* (volume **22**) alone	92 € or $
Back issues of *Alytes*: any single issue	30 € or $
Back issues of *Alytes*: any double issue	50 € or $
Back issues of *Alytes*: any complete volume (4 issues)	80 € or $
Back issues of *Alytes*: complete set volumes **1** to **21**	1260 € or $
SPECIAL OFFER: five-year (2004-2008, volumes **22** to **26**) subscription to *Alytes* + ISSCA + *Circalytes*, with complete set of back issues of *Alytes* (1982-2004, volumes **1** to **21**)	1450 € or $

Circalytes is the internal information bulletin of ISSCA. Back issues of this bulletin are also available: prices can be provided upon request by our Secretariat.

MODES OF PAYMENT

– In Euros, by cheques **drawn on a French bank** payable to "ISSCA", sent to our secretariat (address above).
– In Euros, by direct postal transfer to our postal account: "ISSCA", Nr. 1-398-91 L, Paris; **if you use this mode of payment, add 2.30 € to your payment for postal charges at our end.**
– In US Dollars, by cheques payable to "ISSCA", sent to Rafael O. DE SÁ, Associate Professor, Department of Biology, University of Richmond, Richmond, VA 23173, USA (e-mail: rdesa@richmond.edu; fax: (804)289-8233).

Source : MNHN, Paris

ALYTES

INTERNATIONAL JOURNAL OF BATRACHOLOGY

May 2005 **Volume 22, N° 3-4**

Alytes, 2005, **22** (3-4): 65-71.

The United States Geological Survey's Amphibian Research and Monitoring Initiative

Paul Stephen CORN*, Erin MUTHS** , Michael J. ADAMS***
& C. Kenneth DODD, Jr.****

* US Geological Survey, Aldo Leopold Wilderness Research Institute,
Box 8089, Missoula, Montana 59807, USA

** US Geological Survey, Fort Collins Science Center,
2150 Centre Ave., Bldg C, Fort Collins, Colorado 80526, USA

*** US Geological Survey, Forest and Rangeland Ecosystem Science Center,
3200 SW Jefferson Way, Corvallis, Oregon 97331, USA

**** US Geological Survey, Florida Integrated Science Center,
7920 NW 71st Street, Gainesville, Florida 32653, USA

The Amphibian Research and Monitoring Initiative (ARMI) began in 2000 as an attempt by the United States Geological Survey to determine the status and trends of amphibians on federal lands in the United States and its territories. ARMI research focuses on determining causes of declines, if observed, developing new techniques to sample populations and analyze data, and disseminating information to scientists and policy makers. Monitoring is conducted at multiple scales, with an emphasis on an ability to draw conclusions about status in well-defined study areas such as national parks and wildlife refuges. Several papers originally presented at a national symposium in 2004 are published in this special issue of *Alytes*.

Bibliothèque Centrale Muséum
3 3001 00225906 6

INTRODUCTION

Amphibian decline achieved recognition as a global issue after the meeting of the First World Congress of Herpetology in England in 1989. During the ensuing decade, considerable progress was made in documenting the status of populations and in understanding the causes of some of the declines. However, significant gaps in our knowledge remained, including basic information on status and life history. Additionally, the occurrence of large numbers of malformations in some locations in North America in the mid-1990s increased the urgency to

BIBL. DU MUSÉUM PARIS
Source : MNHN, Paris

critically examine the status of anuran populations. To address these needs, the United States Congress authorized and funded the Amphibian Research and Monitoring Initiative (ARMI) beginning in October 2000. ARMI is a national program coordinated by the United States Geological Survey (USGS) and the science and research bureau for the Department of the Interior (DOI). The goal of ARMI is to better understand the dynamics of amphibian populations, including causes of declines, so that DOI agencies and other land managers have the most accurate information from which to develop effective ways to manage and conserve amphibian populations.

A symposium presenting ARMI monitoring and research results, co-sponsored by the American Society of Ichthyologists and Herpetologists (ASIH) and the International Society for the Study and Conservation of Amphibians (ISSCA), was held at the 2004 joint annual meeting of the three North American herpetological Societies (ASIH, Herpetologists' League and Society for the Study of Amphibians and Reptiles) in Norman, Oklahoma. This issue of *Alytes* presents a sample (6 of 24 papers presented at the symposium) of this work. Prior to introducing these papers, we briefly describe the history, objectives, and basic methods employed by ARMI researchers.

History of ARMI

Herpetology in the USGS came into being when the National Biological Service (NBS) was incorporated into the USGS in 1996. The NBS was a short-lived agency, created only three years before by combining research scientists from the various DOI agencies with land-management responsibilities (primarily the United States Fish and Wildlife Service, National Park Service, and Bureau of Land Management). Several scientists who were employed by these agencies and who now are involved in ARMI have long histories of research on amphibian ecology and conservation. For example, Bury et al. (1980) described the status and conservation issues for a number of amphibians that were either listed as threatened or endangered or were thought to be in need of conservation research. Other examples of studies conducted prior to the First World Congress include Bury (1983), Corn et al. (1989) and Dodd (1991, 1992). In the early 1990s, BRD herpetologists submitted several proposals for broad national or regional surveys, but these were not funded, and there was no coordinated effort among DOI scientists to determine the status and trends of amphibians nationally.

In 1998, the escalating concern over the status of amphibians and the recent discovery of high incidence of developmental malformations in some populations of ranid frogs in the upper Midwest (Meteyer , 2000; Souder, 2000) prompted Bruce Babbitt, then Secretary of the Interior, to request USGS to prepare a budget request for a national amphibian monitoring and research program. This task was performed by a small group of scientists and managers from USGS, the National Park Service, Bureau of Land Management, and US Forest Service during a meeting at Point Reyes National Seashore in June 1998, and funding for amphibian research and monitoring was included in the USGS budget beginning in Fiscal Year 2000. Three USGS Disciplines, Biology, Water and Geography, receive funding through ARMI.

Source : MNHN, Paris

ARMI OBJECTIVES AND METHODS

The goals and methods of ARMI were developed in a series of meetings and workshops by USGS scientists, including an "Amphibian Leadership Team" composed of scientists and managers from USGS and other agencies, largely external to ARMI, which conducted a workshop in Gainesville, Florida in February 2001. The overall goals of ARMI, derived from these meetings (CORN et al., 2005), are to: (1) establish a network designed to monitor the status and changes in the distribution and abundance of amphibian species and communities in the United States; (2) identify environmental conditions known to affect amphibians and document their differences across the Nation; (3) conduct research that identifies causes of amphibian population change and malformations; and (4) provide information to managers, policy makers and the general public in support of amphibian conservation.

The Leadership Team recommended that ARMI adopt a hierarchical approach to monitoring described by the Committee on the Environment and Natural Resources (ANONYMOUS, 1997; BRICKER & RUGGIERO, 1998). This hierarchy can be visualized as a pyramid (fig. 1). At the base, extensive but necessarily coarse measurements are made at many sites across the country. At the apex of the pyramid, intensive research and population monitoring is conducted at a relatively small number of sites throughout the country. At the middle level of the pyramid, monitoring directed toward detecting change in occurrence and abundance of species across the landscape is conducted at a moderate number of sites.

Ideally, the ARMI approach would provide unbiased, base of the pyramid estimates of the status of most amphibians in most habitats across the United States. Realistically, several constraints prevent this approach. Primary among these constraints is the mandate of USGS to provide science support for the other DOI agencies, which for ARMI means devoting the majority of our efforts on lands managed by DOI agencies. Other important constraints are that there are few species distributed widely across the US, that species richness and habitat diversity vary widely among geographic regions, and that amphibians display a variety of reproductive modes and habitat associations. This diversity requires that a variety of sampling methods, rather than a single standardized approach, be used to detect and monitor amphibians across the country, even within regions (HEYER et al., 1994; DODD et al., in press).

For example, the USGS coordinates the North American Amphibian Monitoring Program, an annual volunteer survey of calling frogs in several states in the Midwestern and Eastern United States (MOSSMAN et al., 1998). However, the lack of audible calls by many species, greater aridity of the landscape, sparse road network, and unpredictability of breeding in desert habitats prevents calling surveys from being widely applicable in most of the western United States. Even with standardization, the use of frog call surveys has many limitations associated with sampling representative areas and species detection.

The constraints on collecting base-level data mean that middle-level surveys are the core of ARMI monitoring efforts and are conducted mainly on large protected areas (national parks and wildlife refuges) managed by DOI (HALL & LANGTIMM, 2001). At the middle level of monitoring, ARMI has taken the approach of defining a trend as the change in site

Source : MNHN, Paris

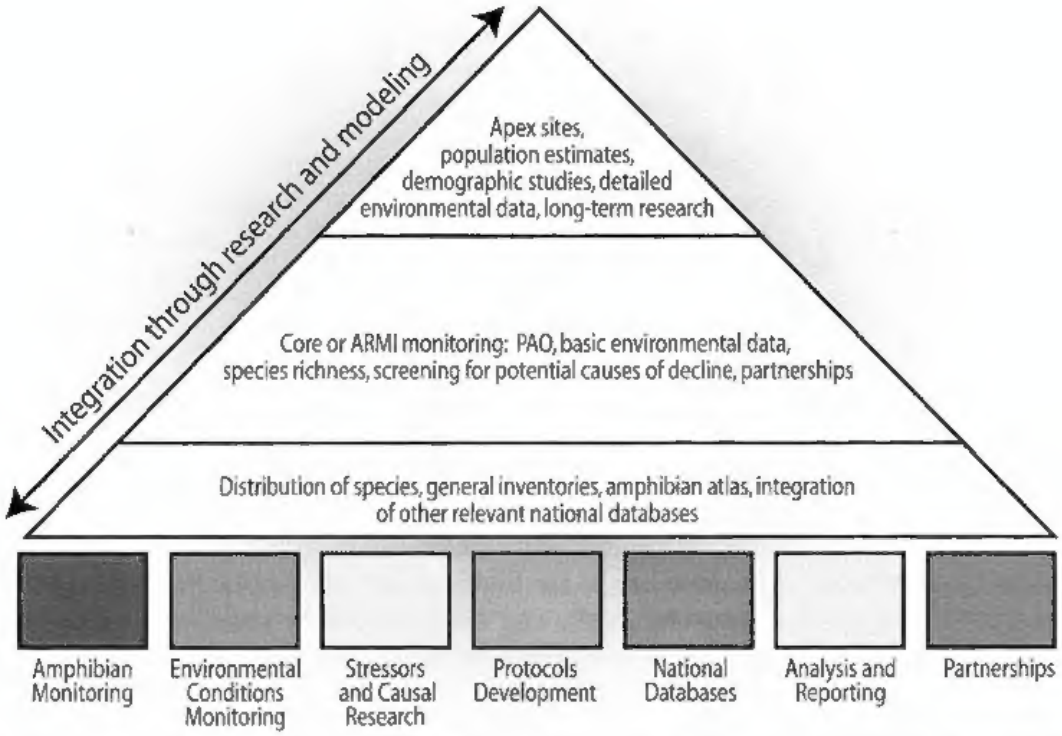


Fig. 1. – The conceptual framework of the United States Amphibian Research and Monitoring Initiative envisioned as a pyramid with three levels. Research and monitoring are integrated across scales, and the pillars across the bottom indicate what is necessary to support a national assessment of amphibian status. See text and HALL & LANGTIMM (2001) and CORN et al. (2005) for additional details.

occupancy by a given species, as recommended by GREEN (1997). For example, ARMI researchers in the mountainous west monitor many lentic-breeding species by documenting change in the proportion of ponds occupied. Other commonly used methods of trend analysis are either impossible to implement on a large scale (direct population estimates) or are unlikely to provide unbiased estimates of change (for example, counts intended to provide an index to true abundance: ANDERSON, 2001; MACKENZIE & KENDALL, 2002; SCHMIDT, 2003). Moreover, changes in occupancy are likely to better reflect amphibian status than changes in abundance for many lentic-breeding species with erratic population dynamics (GREEN, 1997).

Sites are selected for sampling based on a probabilistic scheme to allow inference about status and trend for the defined study area. Because absence in a survey may also indicate failure to detect a species that is actually present, multiple surveys are conducted at sites so that detection probabilities can be calculated and occupancy adjusted to account for errors in detection (MACKENZIE et al., 2002). The approach of monitoring changes in site occupancy of species based on presence/non detection data allows for the estimation of several parameters that can be used to study population and community dynamics, estimate extinction and colonization probabilities, and test hypotheses concerning how environmental factors affect population dynamics. This approach also allows for comparable data to be obtained despite a wide variety of sampling designs. The actual occupancy estimates can only be compared among middle-level monitoring areas to the extent that sites are defined consistently, but

Source : MNHN, Paris

ARMI researchers can compare unbiased estimates of trends in occupancy across the country. Whereas inference is limited to the boundaries of the middle-level monitoring areas, the ARMI approach allows trends to be scaled up to provide regional and national summaries.

Detailed population data are collected by ARMI researchers on a number of species at relatively few locations (apex sites). Unlike middle-level sites, apex sites are not selected randomly, but provide locations for determining demographic and life history characteristics of key species and studying changes in these characteristics over time. Apex monitoring, coupled with controlled manipulations, can sometimes be used for cause and effect hypothesis-testing research.

At all levels of the pyramid, ARMI researchers are encouraged to form partnerships with other agencies, programs and researchers to broaden the scope of investigation beyond DOI. One example is a national amphibian atlas, initiated by Michael Lannoo (LANNOO, 2005) and now hosted by ARMI [http://armi.usgs.gov]. In other cases, middle-level and apex monitoring sites have been established in partnership with other agencies and organizations.

The causes of amphibian declines are varied and can be complex, and ARMI is contributing to understanding both direct and subtle interactions through a number of approaches. Some research is of short-term duration to address a known or suspected problem, but there are still significant gaps in our knowledge of what is causing the declines of many species. A major effort of ARMI includes a multidisciplinary approach to determine environmental factors responsible for the decline or malformation of amphibians.

ARMI SYMPOSIUM

Papers presented at the 2004 symposium and the subset printed in this issue present a sample of work being conducted by USGS scientists and cooperators. For a more complete list of published papers, consult the ARMI web site [http://armi.usgs.gov]. As in the symposium, the papers in this issue reflect a mixture of monitoring and research approaches.

Developing new tools for analysis and refining field methods are ongoing areas of emphasis in ARMI. JUNG et al. compared capture-recapture and removal methods for estimating abundance of stream salamanders in the Appalachian Mountains in Virginia. Removal methods usually resulted in higher capture probabilities for most species, but several sampling episodes are necessary because of high variability among samples.

CORN et al. described a transect of middle-level monitoring sites in the Rocky Mountains along the Continental Divide that includes several of the premier national parks in the United States. Status of amphibians in Colorado at the southern end of the transect is apparently worse than at the northern end in Montana. The southern end of the transect is also characterized by much higher human population and use of park lands, suggesting topics for more focused research on causes of declines.

WENTE et al. surveyed known and random localities for two anurans in the Great Basin in Oregon. Both species were absent from a substantial number of locations where they had

Source : MNHN, Paris

been recorded previously, and present at few new sites. Despite caveats about the effects of prolonged drought in the region, they concluded that at least western toads had likely undergone a recent decline.

In a study related to CORN et al., GREEN & MUTHS surveyed the health status of amphibians in Colorado in and around Rocky Mountain National Park. They found significant levels of infection by chytrid fungus, suggesting the possibility of further declines in this region.

BRIDGES & LITTLE extracted naturally-occurring compounds from amphibian habitats in three national parks or wildlife refuges and assessed their toxicity to developing anuran larvae. The extracts did not cause mortality. However, amphibians reared in extracts had a lengthened larval period or reduced mass at metamorphosis in at least some of the areas studied. Extracts from both the air and water at one site lengthened the larval period. These sublethal effects likely influence life history characteristics which in turn affect population persistence.

Finally, BATTAGLIN et al. demonstrated an important use of the national amphibian atlas. They compiled species richness by county and compared the patterns to climate statistics. As expected, precipitation and temperature were significant variables in explaining richness in most regions. Trends in climate may provide insight into areas of greater stress on amphibian populations.

RÉSUMÉ

L'Amphibian Research and Monitoring Initiative (ARMI) a commencé en 2000. Il s'agit d'une tentative de l'United States Geological Survey de déterminer le statut et l'avenir des amphibiens dans les territoires fédéraux des Etats Unis. Les travaux de l'ARMI sont centrés sur la recherche des causes des déclins, lorsqu'ils existent, la mise au point de nouvelles techniques pour échantillonner les populations et analyser les données, et la diffusion de l'information aux scientifiques et aux décideurs. Les travaux sont conduits à diverses échelles, mais l'accent est particulièrement mis sur la possibilité de tirer des conclusions sur le statut des amphibiens dans des zones d'étude bien définies telles que les parcs nationaux et les réserves naturelles. Plusieurs communications initialement présentées lors d'un symposium aux Etats Unis en 2004 sont publiées dans ce numéro spécial d'*Alytes*.

ACKNOWLEDGMENTS

We thank the ASIH and ISSCA for sponsoring the symposium at the 2004 meetings, and Deanna Stouder, Chair of the ASIH Symposium Committee, for assistance in scheduling the papers in the symposium. Dan James and Rick Kearney, as past and current national coordinators of ARMI, have provided valuable support and guidance throughout the duration of the initiative. We thank the anonymous reviewers of these papers for providing thoughtful comments and insights in a most timely manner. Hanan Enani designed the cover of this special issue under the supervision of Hannah Hamilton. Funding for this special issue of *Alytes* was provided by ARMI.

Source : MNHN, Paris

LITERATURE CITED

ANDERSON, D.R., 2001. – The need to get the basics right in wildlife field studies. *Wildl. Soc. Bull.*, **29**: 1294-1297.

BRICKER, O. P. & RUGGIERO, M. A., 1998. – Toward a national plan for monitoring environmental resources. *Ecol. Applic.*, **8**: 326-329.

BURY, R. B., 1983. – Differences in amphibian populations in logged and old growth redwood forest. *Northwest Sci.*, **57**: 167-178.

BURY, R. B., DODD, C. K., Jr. & FELLERS, G. M., 1980. – Conservation of the Amphibia of the United States: a review. Resource Publications, Washington, US Fish and Wildlife Service, **134**: 1-34.

CORN, P. S., ADAMS, M. J., BATTAGLIN, W. A., GALLANT, A. L., JAMES, D. L., KNUTSON, M., LANGTIMM, C. A., & SAUER, J. R., 2005. – Amphibian research and monitoring initiative: concepts and implementation. *Scientific Investigations Reports*, 2005-5015, Reston, Virginia, US Geological Survey.

CORN, P. S., STOLZENBURG, W. & BURY, R.B., 1989. – Acid precipitation studies in Colorado and Wyoming: interim report of surveys of montane amphibians and water chemistry. *Biological Reports*, Washington, US Fish and Wildlife Service, **80** (40.26): 1-56.

DODD, C.K., Jr., 1991. – The status of the Red Hills salamander *Phaeognathus hubrichti*, Alabama USA, 1976-1988. *Biol. Conserv.*, **55**: 57-75.

----- 1992. – Biological diversity of a temporary pond herpetofauna in north Florida sandhills. *Biodiv. & Conserv.*, **1**: 125-142.

DODD, C. K., Jr., LOMAN, J., COGALNICEANU, D. & PUKY, M., in press. – Monitoring amphibian populations. *In:* H. H. HEATWOLE & J. W. WILKENSON (ed.), *Conservation and decline of amphibians, in: Amphibian biology*, Volume **9A**, Chipping Norton, New South Wales, Australia, Surrey Beatty & Sons Pty. Ltd.

GREEN, D. M., 1997. – Perspectives on amphibian population declines: defining the problem and searching for answers. *In*: D. M. GREEN (ed.), *Amphibians in decline – Canadian studies of a global problem, Herpetological Conservation*, St. Louis, Missouri, Society for the Study of Amphibians and Reptiles, **1**: 291-308.

HALL, R. J. & LANGTIMM, C. A., 2001. – The US national amphibian research and monitoring initiative and the role of protected areas. *George Wright Forum*, **18**: 14-25.

HEYER, W. R., DONNELLY, M. A., MCDIARMID, R. W., HAYEK, L. C. & FOSTER, M. S. (ed.), 1994. – *Measuring and monitoring biological diversity: standard methods for amphibians.* Washington, Smithsonian Institution Press.

LANNOO, M. J. (ed.), 2005. – *Amphibian declines: the conservation status of United States species.* Berkeley, University of California Press, in press.

MACKENZIE, D. I. & KENDALL, W. L., 2002. – How should detection probability be incorporated into estimates of relative abundance? *Ecology*, **83**: 2387-2393.

MACKENZIE, D. I., NICHOLS, J. D., LACHMAN, G. B., DROEGE, S., ROYLE, J. A. & LANGTIMM, C. A., 2002. – Estimating site occupancy rates when detection probabilities are less than one. *Ecology*, **83**: 2248-2255.

METEYER, C., 2000. – Field guide to malformations of frogs and toads, with radiographic interpretations. *Biological Science Reports*, US Geological Survey, USGS/BRD/BSR-2000-0005: 1-18.

MOSSMAN, M. J., HARTMAN, L. M., HAY, R., SAUER, J. R. & DHUEY, B. J., 1998. – Monitoring long-term population trends in Wisconsin frog and toad populations. *In*: M. J. LANNOO (ed.), *Status and conservation of Midwestern amphibians*, Iowa City, University of Iowa Press: 169-198.

SCHMIDT, B. R., 2003. – Count data, detection probabilities, and the demography, dynamics, distribution, and decline of amphibians. *C. r. Biol.*, **326**: S119-S124.

SOUDER, W., 2000. – *A plague of frogs: the horrifying true story*. New York, Hyperion Press.

© ISSCA 2005

Source : MNHN, Paris

Alytes, 2005, **22** (3-4): 72-84.

Estimation of stream salamander (Plethodontidae, Desmognathinae and Plethodontinae) populations in Shenandoah National Park, Virginia, USA

Robin E. JUNG*, J. Andrew ROYLE*, John R. SAUER*, Christopher ADDISON**, Rebecca D. RAU***, Jennifer L. SHIRK**** & John C. WHISSEL*****

* US Geological Survey, Patuxent Wildlife Research Center, 12100 Beech Forest Road, Laurel, Maryland 20708, USA

** 3503 Cascade Loop, Yakima, Washington 98902, USA

*** US Fish and Wildlife Service, Patuxent Research Refuge, 11510 American Holly Drive, Laurel, Maryland 20708, USA

**** Department of Natural Resources, Fernow Hall, Cornell University, Ithaca, New York 14853, USA

***** PO Box 441, Sperryville, Virginia 22740, USA

Stream salamanders in the family Plethodontidae constitute a large biomass in and near headwater streams in the eastern United States and are promising indicators of stream ecosystem health. Many studies of stream salamanders have relied on population indices based on counts rather than population estimates based on techniques such as capture-recapture and removal. Application of estimation procedures allows the calculation of detection probabilities (the proportion of total animals present that are detected during a survey) and their associated sampling error, and may be essential for determining salamander population sizes and trends. In 1999, we conducted capture-recapture and removal population estimation methods for *Desmognathus* salamanders at six streams in Shenandoah National Park, Virginia, USA. Removal sampling appeared more efficient and detection probabilities from removal data were higher than those from capture-recapture. During 2001-2004, we used removal estimation at eight streams in the park to assess the usefulness of this technique for long-term monitoring of stream salamanders. Removal detection probabilities ranged from 0.39 to 0.96 for *Desmognathus*, 0.27 to 0.89 for *Eurycea* and 0.27 to 0.75 for northern spring (*Gyrinophilus porphyriticus*) and northern red (*Pseudotriton ruber*) salamanders across stream transects. Detection probabilities did not differ across years for *Desmognathus* and *Eurycea*, but did differ among streams for *Desmognathus*. Population estimates of *Desmognathus* decreased between 2001-2002 and 2003-2004 which may be related to changes in stream flow conditions. Removal-based procedures may be a feasible approach for population estimation of salamanders, but field methods should be designed to meet the assumptions of the sampling procedures. New approaches to estimating stream salamander populations are discussed.

Source : MNHN, Paris

INTRODUCTION

Stream salamanders including *Desmognathus*, *Eurycea*, *Gyrinophilus* and *Pseudotriton* species in the family Plethodontidae (lungless salamanders) are found in and near seeps and streams in the eastern United States. These salamanders play an important role in nutrient cycling and energy flow and can be top vertebrate predators in fishless headwater streams (DAVIC, 2002). In Appalachian old growth forests, plethodontid salamanders are extremely abundant (up to 18,486 individuals/ha) and constitute a large biomass (16.53 kg/ha) exceeding that of birds (PETRANKA & MURRAY, 2001). Stream salamander larvae develop in seeps and streams. After transformation, juveniles and adults spend part of their life in the leaf litter, rocky substrate and banks along streams, foraging on the surface on wet or humid nights, and hiding beneath rocks, logs, leaves, moss, bark, and in burrows during the day (PETRANKA, 1998).

Because stream salamanders may serve as indicators of stream ecosystem health (CORN & BURY, 1989; PETRANKA et al., 1993, WELSH & OLLIVIER, 1998; SOUTHERLAND et al., 2004), identifying reliable survey methods and population estimation techniques for this group are important. Many studies of stream salamanders have used population indices such as raw counts and densities (WELSH & OLLIVIER, 1998, BARR & BABBITT, 2002), although studies using population estimates based on capture-recapture and removal sampling have also been conducted (BRUCE, 1995; NIJHUIS & KAPLAN, 1998, PETRANKA & MURRAY, 2001). Population estimates that include detection probabilities (the proportion of total animals present that are detected during a survey) and their associated sampling error may be essential in determining population sizes and trends.

Detection probabilities ($\hat{p}$) for salamanders can vary spatially, temporally, and by species and age class (JUNG et al., 2000; SALVIDIO, 2001; BAILEY et al., 2004). If $\hat{p}$'s differ among study sites or over time, population indices will not be comparable unless differences in detection are estimated during sampling. Also, magnitude of detection probability can influence precision and bias of estimated population sizes; if $\hat{p}$'s or recapture rates of animals are low, standard errors of estimated population sizes become large, leading to unreliable or biased estimates.

The purpose of this investigation was two-fold. A study conducted in 1999 was designed to compare capture-recapture and removal estimation methods for stream salamanders and determine which method was more efficient and provided higher detection probabilities. A study conducted from 2001-2004 was meant to assess the usefulness of the preferred technique (removal estimation) for long-term monitoring of stream salamanders in Shenandoah National Park, Virginia, USA.

MATERIALS AND METHODS

For all stream salamander surveys in Shenandoah National Park, we recorded salamander species, age class (those with gills were recorded as larvae and those without were classified

Source : MNHN, Paris

as adults), and snout-vent length (SVL) and total length (in mm). Because larvae of some species were occasionally not distinguished in the field and/or because of low sample sizes, we combined data for northern dusky (*Desmognathus fuscus*) and seal (*D. monticola*) salamanders into *Desmognathus* and combined data for northern spring (*Gyrinophilus porphyriticus*) and northern red (*Pseudotriton ruber*) salamanders into *Gyrinophilus/Pseudotriton* for analyses. The northern two-lined salamander (*Eurycea bislineata*) is found in the northern part of the park, whereas the southern two-lined salamander (*E. cirrigera*) is found in the southern part (GHITEA & SATLER, 1990). Herein, we treat the two species, which are indistinguishable morphologically, as one when streams throughout the park are considered. Stream salamander species in the park not detected in our surveys included the long-tailed (*E. longicauda*) and three-lined (*E. guttolineata*) salamanders (WITT, 1993).

In 1999, we compared capture-recapture and removal methods to estimate stream salamander populations at six streams in the park. Three of the streams were first order (Jeremy's Run, Land's Run, Piney River) and the other three were second order. Two of four observers turned over the top layer of rocks and other objects greater than 6.4 cm maximum width or length within two 50 × 1 m transects (one on each side of the stream channel, 100 m^2 total area) at each stream, sampling the terrestrial habitat immediately adjacent to the wetted stream channel. Larvae were captured in the hyporheic zone and comprised 9 ± 2.8 % (mean ± s_x) of *Desmognathus* observations, 35 ± 8.7 % of *E. bislineata* observations, and 68 ± 11.6 % of *Gyrinophilus/Pseudotriton* observations across all surveys.

Approximately weekly from 9 July to 13 August 1999, we captured salamanders during the day by hand and dipnet and batch marked larvae and adults of or above 25 mm SVL using visible implant fluorescent elastomer (VIE, Northwest Marine Technologies, Inc.), a biocompatible latex-based dye injected just under the skin. We used one of three VIE colors (orange, red, green) at one of four positions just behind the forelimbs or in front of the hindlimbs and checked marks under a blanket using an ultraviolet light (JUNG et al., 2000). Other studies have shown that VIE marks are more permanent and cause salamanders less harm than toe-clipping (DAVIS & OVASKA, 2001; MAROLD, 2001). Identities and measurements of unmarked, marked and recaptured salamanders were recorded at each survey. The percent of salamanders that escaped per stream across capture-recapture surveys averaged 36 ± 2.2 % for *Desmognathus*, 38 ± 5.5 % for *E. bislineata*, and 43 ± 10.3 % for *Gyrinophilus/Pseudotriton*.

After completing 5-6 capture-recapture surveys (five at Keyser Run, Pass Run Tributary, Piney River), we conducted temporary removal sampling of stream salamanders from 23 to 28 August 1999 at the same transects. Three passes, at least two hours apart, were made each day for two consecutive days for a total of six removal passes. We tallied the number of larvae and adults of each species removed at each pass and kept species and age classes in separate buckets with stream water positioned in the stream in the shade. All salamanders were released back to transects after the final pass.

During June and July of 2001-2004, we used temporary removal sampling as above though with 2-3 passes conducted one after the other during the day at 1-2 transects at each of eight streams in the park. Five of the streams were first order and three were second order or higher. Surveys were also conducted at a ninth stream, Staunton River, but no salamanders were detected there, presumably due to residual effects of a large flood along this river in 1995.

Source : MNHN, Paris

For these surveys, we used a modified transect design, transects were 15 m long and 2 m wide and located on only one side of the stream, spanning 1 m along the stream bank and 1 m in the stream channel. This design allowed for capture of significantly more larval *Eurycea* ($F = 21.9$, $df = 1$, $P < 0.001$) and *Gyrinophilus/Pseudotriton* ($F = 4.4$, $df = 1$, $P = 0.043$) salamanders compared to the 1999 stream transects, larvae comprised 5 ± 2.2 % of *Desmognathus* observations, 75 ± 4.1 % of *Eurycea* observations, and 89 ± 4.2 % of *Gyrinophilus/Pseudotriton* observations. Our summer surveys mostly missed *Desmognathus* larvae, which typically transform by June-July (PETRANKA, 1998).

For capture-recapture data from 1999, we estimated population sizes ($\hat{N}$) and detection probabilities ($\hat{p}$) and their standard errors (s_x) using maximum likelihood and Bayesian estimators. These models assume a closed population and fit a series of models that differ in their assumptions about variation in $\hat{p}$ during sampling (OTIS et al., 1978, REXSTAD & BURNHAM, 1991). The test for population closure in program CAPTURE showed that all populations at each stream were closed (all $P > 0.05$), so open population models were not used (REXSTAD & BURNHAM, 1991). At least one assumption of capture-recapture models, that all animals captured are marked, was not met because we estimated only a subset of the populations, i.e., individuals of or above 25 mm SVL. The Bayesian estimator (GAZEY & STALEY, 1986) assumes prior distributions for N and p and estimators are derived based on the posterior distribution. We used a uniform (0,1) prior distribution on p and a diffuse negative binomial prior distribution on N (GEORGE & ROBERT, 1992). We fit the Bayesian model using a Markov chain Monte Carlo technique known as Gibbs sampling, in which the posterior distributions are estimated by simulation. Bayesian estimators do not rely on the asymptotic properties of maximum likelihood estimators, and hence are preferred for small sample sizes (GAZEY & STALEY, 1986).

For removal data from 1999 and 2001-2004, we calculated population estimates using Zippin model M_B (ZIPPIN, 1958; WHITE et al., 1982), which assumes a behavioral response to capture. Escaped salamanders were excluded from removal pass counts. Escapes were higher in 1999 compared to 2001-2004, when the percent of salamanders that escaped per stream across all removal samples averaged 36 ± 3.9 % and 26 ± 3.1 % for *Desmognathus*, 43 ± 7.3 % and 15 ± 1.9 % for *Eurycea*, and 44 ± 18.7 % and 11 ± 3.0 % for *Gyrinophilus/Pseudotriton*, respectively. Basic assumptions for removal studies include population closure, equal sampling effort, equal catchability, and effective reduction of the population after each search. Unfortunately in the case of stream salamanders, some of these assumptions are difficult to meet (BRUCE, 1995).

We used the "closed captures" selection in program MARK (WHITE & BURNHAM, 1999) to calculate N, s_x ($\hat{N}$), $\hat{p}$ and s_x ($\hat{p}$) for capture-recapture model M_0, which assumes a constant capture probability, and removal model M_B. For *Desmognathus* data in 1999, we used paired t tests to test for significant differences between the $\hat{p}$'s of the capture-recapture and removal estimates and used program CONTRAST (SAUER & WILLIAMS, 1989; HINES & SAUER, 1990) to test whether $\hat{p}$'s from the capture-recapture and removal data differed among streams. For the 2001-2004 data, we compared whether $\hat{p}$'s estimated using removal models differed among streams within years and among years within streams for *Desmognathus* and *Eurycea* using a Chi Square test implemented in program CONTRAST. Analyses were conducted within groups (streams or years), then pooled to provide a composite Chi Square test with summed

Source : MNHN, Paris

degrees of freedom. We also tested for differences among years and streams in $\hat{N}$ for *Desmognathus* and *Eurycea* using a two-way ANOVA in SPSS (NORUSIS, 1992)

RESULTS

Across the capture-recapture surveys at the six streams in 1999, we marked 180 *Desmognathus*, 62 *E. bislineata*, and 17 *Gyrinophilus/Pseudotriton* (tab. 1). Recapture rates were fairly low, ranging from 0 to 33 % across species and stream sites (tab. 1). Because the numbers of marked and recaptured salamanders were low, the simplest model (model M_O), which assumes a constant capture probability, was usually the model of choice in program CAPTURE; the results of this model are presented in tab. 2. Unless a population is large or exhibits high capture probabilities, model selection may not be able to detect a pattern in $\hat{p}$'s and will select the default model M_O (MENKENS & ANDERSON, 1988). Because capture-recapture estimates based on maximum-likelihood and Bayesian models were from the same data set, values for $\hat{N}$ and $\hat{p}$ from these methods were quite similar; we used the estimates based on maximum-likelihood for analyses. We were only able to calculate capture-recapture population estimates for *E. bislineata* and *Gyrinophilus/Pseudotriton* at one stream each, Jeremy's Run, where one individual of each species was recaptured (tab. 2). *Desmognathus* individuals were recaptured at all 6 streams and capture-recapture $\hat{p}$'s averaged 0.06 ± 0.014 (range: 0.02-0.10) (tab. 2). Capture-recapture $\hat{p}$'s differed among streams for *Desmognathus* (χ^2 = 13.3, *df* = 5, *P* = 0.02). Capture-recapture does not perform well unless $\hat{p}$'s exceed 0.30 (WHITE et al., 1982), which was never the case. Because of this, the standard errors of $\hat{N}$ were sometimes very large (tab. 2).

Based on the six-pass removal data from 1999, population estimates could be calculated at all 6 stream transects for *Desmognathus*, 3 for *E. bislineata*, and 2 for *Gyrinophilus/Pseudotriton* (tab. 2). Removal $\hat{p}$'s averaged 0.25 ± 0.077 (range: 0.08-0.61) for *Desmognathus* across stream transects, 0.25 ± 0.079 (0.09-0.35) for *E. bislineata*, and 0.44 ± 0.060 (0.38-0.50) for *Gyrinophilus/Pseudotriton*. Removal $\hat{p}$'s differed among streams for *Desmognathus* (χ^2 = 27.9, *df* = 5, *P* < 0.001). Removal $\hat{p}$'s were significantly higher than those based on capture-recapture for *Desmognathus* (*t* = 2.2, *df* = 5, *P* = 0.04, tab. 2).

For the 2001-2004 data, we calculated removal population estimates for stream salamanders at stream transects at the eight streams (tab. 3). Estimation was not possible when zero counts occurred on the second pass when two passes were used or on the second and third passes when three passes were used, or when counts increased across subsequent passes. We could calculate population estimates at 55 %, 54 % and 13 % of the total 56 stream transects surveyed from 2001 to 2004 for *Desmognathus*, *Eurycea* and *Gyrinophilus/Pseudotriton*, respectively (tab. 3). When estimates were calculable, $\hat{p}$'s averaged 0.66 ± 0.025 (range 0.39-0.96) for *Desmognathus* across stream transects, 0.64 ± 0.031 (0.27-0.89) for *Eurycea*, and 0.62 ± 0.065 (0.27-0.75) for *Gyrinophilus/Pseudotriton* (tab. 3). These detection probabilities were much higher than those found in 1999. Using program CONTRAST, we found that $\hat{p}$'s differed among streams for *Desmognathus* (χ^2 = 29.7, *df* = 17, *P* = 0.028) but not for *Eurycea*. Detection probabilities did not differ among years at a stream for either *Desmognathus* or *Eurycea*.

Source : MNHN, Paris

Table 1. – Numbers of salamanders summed across 5 to 6 capture-recapture surveys in 1999 that were too small to mark, escaped capture, or were marked or recaptured (% recaptured in parentheses).

Species	Stream	Not marked	Escaped	Marked	Recaptured (%)
Desmognathus	Jeremy's Run	24	57	56	15 (27)
	Keyser Run	14	29	35	1 (3)
	Land's Run	32	30	28	7 (25)
	North Fork Thornton	15	20	34	2 (6)
	Pass Run Tributary	14	25	18	2 (11)
	Piney River	8	12	9	1 (11)
Eurycea bislineata	Jeremy's Run	7	14	3	1 (33)
	Keyser Run	4	2	2	0 (0)
	Land's Run	4	4	5	0 (0)
	North Fork Thornton	12	20	35	0 (0)
	Pass Run Tributary	12	12	14	0 (0)
	Piney River	3	7	3	0 (0)
Gyrinophilus / Pseudotriton	Jeremy's Run	0	7	8	1 (13)
	Keyser Run	0	1	1	0 (0)
	Land's Run	0	3	1	0 (0)
	North Fork Thornton	0	1	2	0 (0)
	Pass Run Tributary	0	0	1	0 (0)
	Piney River	0	5	4	0 (0)

Table 2. – Population estimates ($N \pm$ standard error, s_x), 95 % confidence intervals (CI), detection probabilities ($\hat{p} \pm s_x$) and models used for species encountered during capture-recapture (CR) and removal (REM) sampling in 1999 at 6 streams in Shenandoah National Park. For Bayesian results, we present the mean N /mode $\hat{N}$.

Species - Stream	Method	n	Passes	$\hat{N}$ (s_x)	95 % CI	$\hat{p}$ (s_x)	Model
Desmognathus							
Jeremy's Run	CR	6		116 (22 5)	86-178	0.10 (0 023)	O
	Bayesian	6		124/119 (25.9)	86-185	0.10 (0.023)	
	REM	6	29,11,13,12,8,7	97 (10.4)	86-131	0.25 (0.053)	B
Land's Run	CR	6		62 (18.8)	41-122	0.09 (0.032)	O
	Bayesian	6		72/66 (25 6)	41-137	0.09 (0 030)	
	REM	6	9,6,5,8,5,4	63 (27 6)	42-180	0 14 (0.085)	B
Pass Run Tributary	CR	5		71 (44.9)	31-242	0.06 (0.038)	O
	Bayesian	5		115/84 (104.9)	32-392	0.06 (0.035)	
	REM	6	12,5,6,6,5,9	108 (90.0)	52-537	0.08 (0.083)	B
Piney River	CR	5		33 (29.4)	13-166	0 06 (0.056)	O
	Bayesian	5		95/47 (159.4)	13-494	0 06 (0.048)	
	REM	6	5,1,4,4,3,0	21 (5.6)	18-48	0 23 (0 117)	B
Keyser Run	CR	5		493 (479 2)	120-2505	0.02 (0.014)	O
	Bayesian	5		556/419 (461.4)	128-1844	0.02 (0.015)	
	REM	6	9,13,1,0,0,0	23 (0.0)	23-23	0.61 (0.079)	B
North Fork Thornton	CR	6		253 (169.3)	91-872	0.02 (0.016)	O
	Bayesian	6		346/266 (275.5)	97 1074	0.03 (0.0.6)	
	REM	6	11,14,2,11,4,6	70 (18.3)	53-139	0.17 (0.072)	B
Eurycea bislineata							
Jeremy's Run	CR	6		4 (2.7)	3-20	0 17 (0.139)	O
	Bayesian	6		19/7 (60 1)	3-110	0 12 (0.095)	
	REM	6	6,2,2,5,4,3	50 (51 3)	25-323	0.09 (0.115)	B
Pass Run	REM	6	1,2,0,3,0,0	6 (1 1)	6-6	0.35 (0.116)	B
Piney River	REM	6	1,0,0,1,1,0	3 (0.0)	3-3	0.30 (0.145)	B
Gyrinophilus Pseudotriton							
Jeremy's Run	CR	6		27 (24 2)	11-137	0 06 (0.051)	O
	Bayesian	6		82/40 (138.4)	11-433	0 05 (0.043)	
North Fork Thornton	REM	6	0,1,0,0,0,0	1 (0 0)	1-1	0 50 (0 354)	B
Piney River	REM	6	1,0,1,1,0,0	3 (0.0)	3-3	0.38 (0 171)	B

Source : MNHN, Paris

Table 3. Population estimates ($\hat{N} \pm s_{\hat{N}}$), 95 % confidence intervals (CI) and detection probabilities ($\hat{p} \pm s_{\hat{p}}$) for salamanders encountered during removal sampling in Shenandoah National Park. Analyses were based on two or three diurnal passes conducted consecutively at one (2001) or two (2002-04) transects (T) at each of eight streams (escaped salamanders excluded) using model M_B (Zippin) from program CAPTURE. – indicates a third pass was not conducted. No data for a particular species for a year, stream or transect indicates that none were detected or that estimation was not possible.

Taxon - Stream	Year	T	Passes 1 2,3	$\hat{N}$ ($s_{\hat{N}}$)	95% CI	$\hat{p}$ ($s_{\hat{p}}$)
Desmognathus						
Piney River	2004	1	1 1.0	2 (0.38)	2-2	0.67 (0.272)
Piney Tributary	2002	1	3.0.1	4 (0.54)	4-4	0.67 (0.192)
		2	4 2.2	9 (1.86)	9-20	0.52 (0.245)
	2003	1	2 1 -	3 (0.75)	3-3	0.75 (0.217)
	2004	1	2 1 0	3 (0.27)	3-3	0.75 (0.217)
		2	2,1.0	3 (0.27)	3-3	0.75 (0.217)
Ivy Creek	2001	1	19.12.-	44 (14.98)	34-109	0.45 (0.207)
	2002	1	31 4.-	35 (0.75)	35-35	0.90 (0.049)
		2	29.12.-	47 (5.80)	43-70	0.63 (0.129)
	2003	1	6.4.-	12 (3.85)	11-34	0.58 (0.297)
		2	1,1.0	2 (0.38)	2-2	0.67 (0.272)
	2004	1	3.3.2	10 (4.30)	9-34	0.39 (0.285)
		2	7.0.2	9 (0.69)	9-9	0.69 (0.128)
Doyle's River	2001	1	9,1.-	10 (0.35)	10-10	0.91 (0.087)
	2002	2	7 5 0	12 (0.73)	12-12	0.71 (0.111)
	2004	1	5,1.2	8 (1.06)	8-8	0.62 (0.135)
Hawksbill	2001	1	11.8,-	28 (14.37)	21-100	0.42 (0.278)
	2002	1	24.1,-	25 (0.21)	25-25	0.96 (0.038)
	2003	2	1,2 0	3 (0.71)	3-3	0.60 (0.219)
	2004	1	2,0 1	3 (0.71)	3-3	0.60 (0.219)
		2	6,1.1	8 (0.51)	8-8	0.73 (0.134)
Pass Run	2002	2	6.1 2	9 (0.95)	9-9	0.64 (0.128
	2004	1	3.4.0	7 (0.87)	7-7	0.64 (0.145)
		2	4.2.1	7 (0.87)	7-7	0.64 (0.145)
Jeremy's Run	2001	1	8 4,-	13 (2.41)	13-27	0.68 (0.224
	2002	1	9.4.4	20 (4.15)	18-40	0.46 (0.177)
		2	4 2.0	6 (0.38)	6-6	0.75 (0.153)
	2003	1	13.3.-	16 (0.88)	16-16	0.84 (0.084)
		2	7,5,-	15 (6.61)	13-51	0.51 (0.307)
	2004	1	10.6.0	16 (0.72)	16-16	0.73 (0.095)
		2	7,2.1	10 (0.63)	10-10	0.71 (0.121)
Eurycea						
Piney River	2002	2	10.6.1	17 (1.04)	17-22	0.68 (0.13)
	2003	2	1 1.0	2 (0.38)	2-2	0.66 (0.272
	2004	1	5.4.2	12 (2.74)	12-28	0.49 (0.215)
Piney Tributary	2001	1	14.7.-	24 (4.97)	22-48	0.60 (0.193)
	2002	2	8.11.4	34 (14.05)	25-98	0.31 (0.183)
	2003	1	17.6,-	24 (2.47)	24-37	0.73 (0.141)
		2	12.7,-	24 (6.77)	20-56	0.54 (0.228)
	2004	1	9 1.2	12 (0.73)	12-12	0.71 (0.111)
		2	5.4.1	10 (1.11)	10-10	0.62 (0.121)
Ivy Creek	2001	1	7 1 -	8 (0.40)	8-8	0.89 (0.105)
Doyle's River	2003	1	4 2.-	6 (1.05)	6-6	0.75 (0.153)
	2004	1	6,3.1	10 (0.86)	10-10	0.67 (0.122)
Hawksbill	2001	1	6 3 -	9 (1.43)	9-18	0.74 (0.232)
	2002	1	18.4.-	22 (1.00)	22-22	0.85 (0.07)
	2003	1	4 2.-	6 (1.05)	6-6	0.75 (0.153)
	2004	1	3 1.2	6 (1.69)	6-17	0.51 (0.294)
		2	18.2.1	21 (0.32)	21-21	0.84 (0.073)
Pass Run	2002	2	12 8 4	28 (4.59)	25-48	0.47 (0.146)
	2004	1	10 4.6	27 (9.65)	21-72	0.35 (0.187)
		2	14 9 7	36 (11.15)	32-87	0.36 (0.15)
Jeremy's Run	2001	1	6 2.-	8 (0.87)	8-8	0.80 (0.177)
	2002	1	5 1 2	8 (1.06)	8-8	0.62 (0.135)
	2004	1	12 3 3	18 (1.32)	18-27	0.65 (0.135)
		2	5 2.0	7 (0.33)	7-7	0.78 (0.139)
Paine Run	2001	1	29 23 -	112 (73.11)	62-447	0.27 (0.206
	2002	1	16 9 -	32 (8.27)	26-69	0.53 (0.202
		2	117 21 -	146 (3.56)	143-158	0.81 (0.044
	2003	2	21 9	34 (4.84)	31-55	0.64 (0.15
	2004	1	5 0	6 (0.14)	6-6	0.86 (0.132
		2	3 3 0	6 (0.67)	6-6	0.67 (0.157
Gyrinophilus / *Pseudotriton*						
Ivy Creek	2002	2	4 2 -	6 (1.05)	6-120	0.75 (0.153)
Doyle's River	2002	1	6 7 4	27 (16.50)	19-110	0.37 (0.223)
		2	5 0 1	6 (0.38)	6-6	0.75 (0.153)
Jeremy's Run	2004	1	2 2 0	4 (0.54)	4-4	0.67 (0.192)
Paine Run	2002	1	3 2 -	5 (1.20)	5-5	0.71 (0.177)
	2004	1	10 5 3	19 (2.50)	19-32	0.54 (0.154)
		2	2 2 0	4 (0.54)	4-4	0.67 (0.192)

Source : MNHN, Paris

To analyze population change at a site, at least two years of data are needed and analyses should rely on population estimates to avoid bias associated with raw counts. For *Desmognathus* and *Eurycea*, we had complete sets of $\hat{N}$ for 2001-2004 at three streams each and $\hat{N}$ for 3 of the 4 years at another two streams (tab. 3). We found significant differences in $\hat{N}$ across years ($F = 12.7$, $df = 3,9$, $P = 0.001$) and streams ($F = 9.9$, $df = 4,9$, $P = 0.002$) as well as a significant year*stream interaction ($F = 4.6$, $df = 10,9$, $P = 0.015$) for *Desmognathus*, but no significant differences for *Eurycea*. *Desmognathus* population estimates were significantly higher in 2001 (24 ± 7.8) and 2002 (20 ± 5.4) compared to 2003 (9 ± 2.7) and 2004 (8 ± 1.4). The park experienced heavy precipitation during the summer of 2003, with average stream flow rates 19 and 7 times higher than stream flow rates in the summers of 2002 and 2001, respectively (Shenandoah Watershed Study data, Rick Webb, pers. comm.). Data from July 2004 are not yet available, but 2004 flow rates were most likely intermediate between the 2003 and 2001-2002 flow rates.

DISCUSSION

Long-term monitoring programs require cost-effective and efficient techniques to gather accurate and precise data. Unfortunately, the spatially variable (i.e., significant differences among streams) and sometimes low detection probabilities found in this study using capture-recapture and removal methods reinforce the need for estimating $\hat{p}$'s as part of stream salamander abundance estimation studies. Our study also indicates the importance of developing better methods for estimating stream salamander populations such that estimates are consistently available on a yearly basis for trend analyses.

We found that removal sampling yielded higher $\hat{p}$'s for stream salamanders than capture-recapture sampling. Other capture-recapture surveys of stream salamanders have also shown low recapture rates and hence detection probabilities (BARTHALMUS & BELLIS, 1972, NIJHUIS & KAPLAN, 1998). Indeed, MAROLD (2001) used VIE to mark 44 *E. bislineata* and *D. fuscus* but did not recapture any in the field. BRUCE (1995) used removal sampling (7 passes set 2-3 days apart) and found low to moderate standard errors for population estimates of *D. monticola* and suggested removal sampling was a promising technique to monitor salamander demographics. Other factors favoring removal over capture recapture sampling are that removal sampling usually requires shorter sampling intervals, reduced field personnel, and less funding than capture-recapture, and appears to be ideal for amphibians such as aquatic larvae that are highly detectable and have limited home ranges and mobility (HAYEK, 1994).

If removal sampling is to be used for long-term monitoring, field protocols play an important role in determining their success. In our removal surveys, effective reduction of populations sometimes did not occur even after six passes. This may be due in part to the high percentage of salamanders that escaped capture, though if we analyzed removal data including escapes in the passes, the same issues would be apparent. It is important to note that when the percent of escapes were lower as they were in 2001-2004 compared to 1999, the $\hat{p}$'s for species were higher. With fewer escapes and larger sample sizes, there is potential for better estimates. Removal estimates using Zippin's method are unreliable if less than half the

Source : MNHN, Paris

population is removed (BRUCE, 1995), but WHITE et al. (1982) considered detection probabilities greater than 0.20 adequate for estimating population abundance in removal experiments. BRUCE (1995) found that 7 passes probably reduced total *D. monticola* populations by more than half at his study sites, but he had difficulties reducing numbers of first year juveniles, which may have shown "increased surface activity ..as the larger salamanders were removed (i.e., a response to reduced competition or predation)". SOUTHERLAND et al. (2004) used two-pass removal sampling and were unable to calculate population estimates for species at an average of 75 % of the streams surveyed because salamander numbers did not decrease or were zero in the second pass. Removing salamanders from under the top layer of rocks may disturb or "unearth" other salamanders deeper in the rock substrate. As we sometimes observed, this can lead to more salamanders in the surface population during subsequent passes than in the first pass before disturbance.

Several factors could be changed in our removal protocol to improve $\hat{N}$ and $\hat{p}$ estimates. Conducting surveys on wet or humid nights, when more of the salamander population may be on the surface foraging, might yield better removal estimates. *D. fuscus* and *E. bislineata* emerge one hour after sunset (HOLOMUZKI, 1980) and *D. monticola* emerge shortly after dark, with peak activity occurring around midnight and again at dawn (SHEALY, 1975; HAIRSTON, 1986). However, working at night along rocky streams can be difficult and treacherous. Another option would be to conduct more removal passes, providing the option to group data from earlier passes in which no decreases in removals occurred. Pilot studies in which a large number of removal passes are conducted to determine the appropriate number and grouping of passes may be useful. Another factor to consider is the size and placement of transects or plots. In our surveys, we only searched narrow 1- or 2-m bands along and/or in the stream. Most stream salamanders move between the stream channel, splash zone and bank. Home ranges of *D. fuscus* have been shown to vary tremendously, from 1.4 m^2 in Ohio (ASHTON, 1975) to 25-114 m^2 in Kentucky (BARBOUR et al., 1969). *D. monticola* home ranges were estimated to be 8.4 m^2 in Kentucky (HARDIN et al., 1969). During warm months, *E. bislineata* tagged with radioactive isotopes moved within a 14 m^2 area (ASHTON & ASHTON, 1978), but in June some post-breeding migrants moved more than 100 m from a stream (MACCULLOCH & BIDER, 1975), which probably explains the particularly low recaptures we observed for this species in the 1999 capture-recapture surveys. Surveying a wider area of bank along with the stream channel to incorporate more of the target species' individual home ranges may yield better removal estimates.

Other new approaches may prove to be more useful for stream salamander population estimation. Our removal estimates were based on populations at single stream transects. New analytical methods developed by ROYLE (2004*a*-*b*), ROYLE et al. (2004) and DORAZIO et al. (in press) aggregate information across sample sites such that removal sampling can estimate the abundance of spatially distinct subpopulations. These models incorporate spatial models of abundance (e.g., Poisson, negative binomial) with models of detection probability and have been shown to yield abundance estimates with "similar or better precision than those computed using the conventional approach of analyzing the removal counts of each subpopulation separately" (DORAZIO et al., in press).

A different approach would be to estimate the proportion of area (in this case, streams) occupied (PAO) by stream salamanders over time (MACKENZIE et al., 2002). The PAO method

Source : MNHN, Paris

estimates site occupancy and detectability of species based on presence/absence data recorded from repeated visits to sites selected using a probabilistic sampling frame within an area of inference. Stream salamander species that exhibit low detection probabilities and occupy fewer sites would require more streams and visits per stream for PAO estimation (MACKENZIE & ROYLE, submitted). Note that repeated visits to streams could be satisfied by surveying multiple transects along the length of a stream.

Despite the problems evident in this study, population estimation efforts incorporating detection probabilities may be necessary to assess trends in stream salamander populations. Better survey methods (e.g., transect designs) and population estimation techniques (e.g., aggregated removal or PAO approaches) need to be tested and developed such that reasonably low bias population estimates can be consistently calculated for sites over time. In addition, spatial design of sampling associated with hypothesis testing incorporating covariates that may influence stream salamanders ($\hat{p}$, $\hat{N}$, site occupancy), such as the percent of impervious surface in a watershed and stream flow rates, should be incorporated alongside monitoring to best yield inferences about how changes in stream salamander populations over time are influenced by environmental factors.

RÉSUMÉ

Dans l'est des États-Unis, les salamandres torrenticoles de la famille des Plethodontidae représentent une biomasse élevée dans et auprès des ruisseaux issus des sources. Elles peuvent ainsi constituer d'intéressants indicateurs de la santé de ces écosystèmes. Beaucoup d'etudes de ces salamandres se sont appuyees sur des indices démographiques utilisant des décomptes d'animaux et non pas sur des estimations fondées sur des techniques comme les captures-recaptures ou le ramassage des individus. L'emploi de procédures d'evaluation permet le calcul de probabilités de détection (la proportion d'animaux réellement presents détectés lors d'une etude) et de leur ecart-type, et peut permettre de déterminer les tailles et les dynamiques des populations de salamandres. En 1999, nous avons employé les méthodes de capture-recapture et de ramassage pour évaluer des populations de salamandres du genre *Desmognathus* dans six ruisseaux du Parc National de Shenandoah (Virginie, Etats-Unis). La méthode du ramassage s'est avérée plus efficace: elle a donné des probabilites de détection plus élevees que celle de capture-recapture. Pendant la période 2001-2004, nous avons employé la methode du ramassage dans et auprès de huit ruisseaux du Parc afin d'évaluer la fiabilite de cette technique pour la surveillance à long terme de ces populations de salamandres. Lors de transects le long des ruisseaux, nous avons obtenu des probabilites de detection de 0,39 à 0,96 pour *Desmognathus*, de 0,27 à 0,89 pour *Eurycea* et de 0,27 à 0,75 pour *Gyrinophilus porphyriticus*/*Pseudotriton ruber*. Les probabilités de détection n'ont pas varié au cours des annees pour *Desmognathus* et *Eurycea*, mais ont différé selon les ruisseaux pour *Desmognathus*. Les evaluations des populations de *Desmognathus* ont diminue entre 2001-2002 et 2003-2004, ce qui peut être lié à des changements dans le régime hydrique des ruisseaux. Les procedures de ramassage constituent une methode fiable pour l'evaluation de populations de ces salamandres, mais les méthodes de terrain doivent être conçues de manière à remplir les

Source : MNHN, Paris

conditions statistiques des méthodes d'échantillonnage. De nouvelles méthodes d'estimation des populations de ces salamandres sont discutées.

Acknowledgments

We would like to thank James Atkinson at Shenandoah National Park, the 2001-2004 field crews (Isaac Chellmann, Sara Faust, Lindsay Funk, Evan H. C. Grant, Andrew Mongeon, Priya Nanjappa, Sheera Schneider, Edward Schwartzman), and Sam Droege. This work was funded by the Park Research and Intensive Monitoring of Ecosystems Network (PRIMENet), a joint initiative of the National Park Service and Environmental Protection Agency, and by the US Geological Survey's Amphibian Research and Monitoring Initiative (ARMI).

Literature cited

Ashton, R. E., Jr., 1975. A study of movement, home range, and winter behavior of *Desmognathus fuscus* (Rafinesque). *J. Herp.*, **9**: 85-91.

Ashton, R. E., Jr. & Ashton, P. S., 1978. Movements and winter behavior of *Eurycea bislineata* (Amphibia, Urodela, Plethodontidae). *J. Herp.*, **12**: 295-298.

Bailey, L. L., Simons, T. R. & Pollock, K. H., 2004. Spatial and temporal variation in detection probability of *Plethodon* salamanders using the robust capture-recapture design. *J. Wildl. Management*, **68**: 14-24.

Barbour, R. W., Hardin, J. W., Schafer, J. P. & Harvey, M. J., 1969. Home range, movements, and activity of the dusky salamander, *Desmognathus fuscus*. *Copeia*, **1969**: 293-297.

Barr, G. E. & Babbitt, K. J., 2002. Effects of biotic and abiotic factors on the distribution and abundance of larval two-lined salamanders (*Eurycea bislineata*) across spatial scales. *Oecologia*, **133**: 176-185.

Barthalmus, G. T. & Bellis, E. D., 1972. Home range, homing, and the homing mechanism of the salamander, *Desmognathus fuscus*. *Copeia*, **1972**: 632-642.

Bruce, R. C., 1995. The use of temporary removal sampling in a study of population dynamics of the salamander *Desmognathus monticola*. *Austr. J. Ecol.*, **20**: 403-412.

Corn, P. S. & Bury, R. P., 1989. Logging in western Oregon: responses of headwater habitats and stream amphibians. *Forest Ecol. Management*, **29**: 39-57.

Davic, R. D. (ed.), 2002. *Field evaluation manual for Ohio's primary headwater habitat streams*. Version 1.0. Columbus, Ohio, USA, Ohio Environmental Protection Agency, Division of Surface Water: 1-37.

Davis, T. M. & Ovaska, K., 2001. Individual recognition of amphibians: effects of toe clipping and fluorescent tagging on the salamander *Plethodon vehiculum*. *J. Herp.*, **35**: 217-225.

Dorazio, R. M., Jelks, H. L. & Jordan, F., in press. Improving removal-based estimates of abundance by sampling a population of spatially distinct subpopulations. *Biometrics*.

Gazey, W. J. & Staley, M. J., 1986. Population estimation from mark-recapture experiments using a sequential Bayes algorithm. *Ecology*, **67**: 941-951.

Giorgi, E. I. & Robert, C. P., 1992. Capture-recapture estimation via Gibbs sampling. *Biometrika*, **79**: 677-683.

Ghitea, O. & Sattler, P., 1990. The distribution and identification of two-lined salamanders in Virginia. *Catesbeiana*, **10**: 11-18.

Hairston, N. G., Sr., 1986. – Species packing in *Desmognathus* salamanders: experimental demonstration of predation and competition. *Am. Nat.*, **127**: 266-291.

Hardin, J. W., Schafer, J. P. & Barbour, R. W., 1969. Observations on the activity of a seal salamander, *Desmognathus monticola*. *Herpetologica*, **25**: 150-151.

Source : MNHN, Paris

HAYEK, L. C., 1994. Removal sampling. *In* W. R. HEYER, M. A. DONNELLY, R. W. MCDIARMID, L. C. HAYEK & M. S. FOSTER (ed.), *Measuring and monitoring biological diversity: standard methods for amphibians*, Washington, DC, Smithsonian Institution Press. 201-205.

HINES, J. E. & SAUER, J. R., 1990. Program CONTRAST: a general program for the analysis of several survival or recovery rate estimates. *US Department of the Interior, Fish and Wildlife Service Technical Reports*, **24**: 1-7.

HOLOMUZKI, J. R., 1980. Synchronous foraging and dietary overlap of three species of plethodontid salamanders. *Herpetologica*, **36**: 109-115.

JUNG, R. E., DROEGE, S., SAUER, J. R. & LANDY, R. B., 2000. Evaluation of terrestrial and streamside salamander monitoring techniques at Shenandoah National Park. *Environ. Monit. Assessment*, **63**: 65-79.

MACCULLOCH, R. D. & BIDER, J. R., 1975. Phenology, migrations, circadian rhythm and the effect of precipitation on the activity of *Eurycea b. bislineata* in Quebec. *Herpetologica*, **31**: 433-439.

MACKENKIE, D. I., NICHOLS, J. D., LACHMAN, G. B., DROEGE, S., ROYLE, J. A. & LANGTIMM, C. A., 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology*, **83**: 2248-2255.

MACKENZIE, D. I. & ROYLE, J. A., submitted. Designing occupancy studies: general advice and tips on allocation of survey effort. *J. appl. Ecol.*

MAROLD, M. R., 2001. - Evaluating visual implant elastomer polymer for marking small, stream-dwelling salamanders. *Herp. Rev.*, **32**: 91-92.

MENKENS, G. E., Jr & ANDERSON, S. H., 1988. - Estimation of small-mammal population size. *Ecology*, **69**: 1952-1959.

NIJHUIS, M. J. & KAPLAN, R. H., 1998. - Movement patterns and life history characteristics in a population of the cascade torrent salamander (*Rhyacotriton cascadae*) in the Columbia River gorge, Oregon. *J. Herp.*, **32**: 301-304.

NORUSIS, M. J., 1992. - *SPSS/PC+ Base System User's Guide Version 5.0*. Chicago, Il., SPSS Inc.

OTIS, D. L., BURNHAM, K. P., WHITE, G. C. & ANDERSON, D. R., 1978. - Statistical inference from capture data on closed animal populations. *Wildlife Monogr.* **62**: 1-135.

PETRANKA, J. W., 1998. - *Salamanders of the United States and Canada*. Washington, DC, Smithsonian Institution Press: 1-587.

PETRANKA, J. W., ETHERIDGE, M. E. & HALEY, K. E., 1993. - Effects of timber harvesting on southern Appalachian salamanders. *Conserv. Biol.*, **7**: 363-370.

PETRANKA, J. W. & MURRAY, S. S., 2001. Effectiveness of removal sampling for determining salamander density and biomass: a case study in an Appalachian streamside community. *J. Herp.*, **35**: 36-44.

REXSTAD, E. & BURNHAM, K., 1991. *User's guide for interactive program CAPTURE. abundance estimation of closed animal populations*. Fort Collins, Colorado, USA, Colorado Cooperative Fish & Wildlife Research Unit, Colorado State University: 1-29.

ROYLE, J. A., 2004*a*. N-mixture models for estimating population size from spatially replicated counts. *Biometrics*, **60**: 108-115.

----- 2004*b*. Generalized estimators of avian abundance from count survey data. *Anim. Biodiv. Conserv.*, **27**: 375-386.

ROYLE, J. A., DAWSON, D. K. & BATES, S., 2004. Modeling abundance effects in distance sampling. *Ecology*, **85**: 1591-1597.

SALVIDIO, S., 2001. Estimating terrestrial salamander abundance in different habitats: efficiency of temporary removal methods. *Herp. Rev.*, **32**: 21-24.

SAUER, J. R. & WILLIAMS, B. K., 1989. Generalized procedures for testing hypotheses about survival or recovery rates. *J. Wildl. Management*, **53**: 137-142.

SHEALY, R. M., 1975. Factors influencing activity in the salamanders *Desmognathus ochrophaeus* and *D. monticola* (Plethodontidae). *Herpetologica*, 31: 94-102.

SOUTHERLAND, M. T., JUNG, R. E., BAXTER, D. P., CHELLMANN, I. C., MERCURIO, G. & VOLSTAD, J. H., 2004. Stream salamanders as indicators of stream quality in Maryland, USA. *Appl. Herp.*, **2**: 23-46.

WELSH, H. H., Jr & OLLIVIER, L. M., 1998. Stream amphibians as indicators of ecosystem stress: a case study from California's Redwoods. *Ecol. Appl.*, **8**: 1118-1132.

WHITE, G. C. & BURNHAM, K. P., 1999. Program MARK: survival estimation from populations of marked animals. *Bird Study*, **46**: 120-138.

Source : MNHN, Paris

WHITE, G. C., ANDERSON, D. R., BURNHAM, K. P. & OTIS, D. L., 1982. *Capture-recapture and removal methods for sampling closed populations.* Los Alamos, New Mexico, USA, Los Alamos National Laboratory LA-8787-NERP: 1-235.

WITT, W. L., 1993. Annotated checklist of the amphibians and reptiles of Shenandoah National Park, Virginia. *Catesbeiana*, **13**: 26-35.

ZIPPIN, C., 1958. The removal method of population estimation. *J. Wild. Management*, **22**: 82-90.

Corresponding editor: C. Kenneth DODD, Jr.

© ISSCA 2005

Source : MNHN, Paris

Status of amphibians on the Continental Divide: surveys on a transect from Montana to Colorado, USA

Paul Stephen CORN*, Blake R. HOSSACK*, Erin MUTHS**, Debra A. PATLA***, Charles R. PETERSON*** & Alisa L. GALLANT****

* US Geological Survey, Aldo Leopold Wilderness Research Institute, Box 8089, Missoula, Montana 59807, USA

** US Geological Survey, Fort Collins Science Center, 2150 Centre Ave., Bldg C, Fort Collins, Colorado 80526, USA

*** Department of Biological Sciences, Idaho State University, Campus Box 8007, Pocatello, Idaho 83209, USA

**** US Geological Survey, EROS Data Center, Sioux Falls, South Dakota 57198, USA

The Rocky Mountain Region of the United States Geological Survey's Amphibian Research and Monitoring Initiative is conducting monitoring of the status of amphibians on a transect that extends along the Continental Divide from Canada to Colorado and comprises four National Parks. Monitoring uses visual encounter surveys to determine site occupancy, with multiple visits to a subset of sites to estimate detection probabilities for each species. Detection probabilities were generally high (above 0.65) among species. There was a gradient in site occupancy, with most species scarce in the south and relatively common in the north. For example, *Bufo boreas* is close to extinction in Rocky Mountain National Park, was found at fewer than 5 % of sites in Yellowstone and Grand Teton National Parks in the middle of the transect, but occurs at approximately 10 % of sites in Glacier National Park. The salamander *Ambystoma tigrinum was rare in Rocky Mountain and occurred at less than 25 % of sites at Yellowstone and Grand Teton, but A. macrodactylum* occurred at more than 50 % of sites in Glacier. There are numerous differences among parks, such as latitude, climate, numbers of visitors, and human population density in the surrounding landscape. The degree to which these factors have influenced the current distribution and abundance of amphibians is unknown but should be a focus of additional research.

INTRODUCTION

Amphibian declines have occurred in the western United States at a disproportionate rate (BRADFORD, 2005). These declines are not restricted to areas where land use has directly

Source : MNHN, Paris

altered habitat, but have occurred on protected federal lands including national parks and wilderness areas (DROST & FELLERS, 1996; KNAPP & MATTHEWS 2000; MATTHEWS et al , 2003, MUTHS et al., 2003). To determine the status and trends of amphibian species in the Rocky Mountains, which contain some of the United States' most significant protected landscapes, the Rocky Mountain Region of the United States Geological Survey Amphibian Research and Monitoring Initiative (ARMI) has established a transect along the Continental Divide that includes four national parks as middle-level monitoring sites. The middle level refers to a pyramid of monitoring efforts, starting with base-level inventories over large geographic areas and ending with focused apex studies at a few sites (HALL & LANGTIMM, 2001; CORN et al., 2005). At the middle level, surveys are conducted within a defined study area, such as a national park, with a sampling design that allows inference about the status and trends of the amphibians within that area.

The national parks on the Continental Divide are spaced across about 8° of latitude, from Rocky Mountain in Colorado, through Grand Teton and Yellowstone in northwest Wyoming (referred to collectively as GYE for brevity and because they compose the center of the Greater Yellowstone Ecosystem), to Glacier in northwest Montana (fig. 1). The parks differ in size, climate, and potential degree of anthropogenic influence (tab. 1). All three study areas are characterized by similar zones of vegetation At lower elevations, the coniferous montane forest is dominated by ponderosa pine (*Pinus ponderosa*), lodgepole pine (*P contorta*) or Douglas fir (*Pseudotsuga menziesii*), with western redcedar (*Thuja plicata*) and western larch (*Larix occidentalis*) in Glacier The mid elevation subalpine forests are composed primarily of Engelmann spruce (*Picea engelmannii*), subalpine fir (*Abies lasiocarpa*) and white pines (*Pinus flexilis*, *P albicaulis*) There are also significant alpine habitats above tree line, which decreases about 100 m in elevation for every 1° north in latitude, from about 3500 m in Rocky Mountain to 2600 m in Glacier (PEET, 1988).

Amphibian declines have occurred to the greatest extent at the southern end of the transect In Rocky Mountain National Park, the boreal toad (*Bufo boreas*) is nearing extinction (MUTHS et al , 2003) and the northern leopard frog (*Rana pipiens*) has not been observed since 1974 (CORN et al , 1997) At the northern end of the transect, surveys in Glacier have found all of the Park's resident amphibian species, and there is little suggestion of recent declines (MARNELL, 1997; ADAMS et al , 2005; PSC & BRH, unpublished). Surveys in the 1990s and in 2000 and 2001 suggest that amphibian declines in the two parks of the GYE may be intermediate between Rocky Mountain and Glacier *Rana pipiens* has largely disappeared from the GYE, and *B boreas* is present, but at a reduced number of locations (KOCH & PETERSON, 1995, VAN KIRK & PATLA, 2000; PATLA & PETERSON, 2001*a-b*).

Conducting surveys to determine status and trends of amphibians in all three study areas (Glacier, GYE, Rocky Mountain) provides the ability to monitor changes over an unprecedented latitudinal gradient and the opportunity to compare changes in status of amphibians to gradients in climate and habitat. Given the erratic nature of the population dynamics of many amphibian species, ARMI has chosen to follow the advice of GREEN (1997) and concentrate on detecting change in numbers of populations rather than numbers of individuals within populations (CORN et al , 2005). We determine status of each species by using the presence or absence of breeding at individual sites to estimate occupancy, expressed as the proportion of sites occupied Trends will be assessed by examining changes in occupancy once

Source : MNHN, Paris

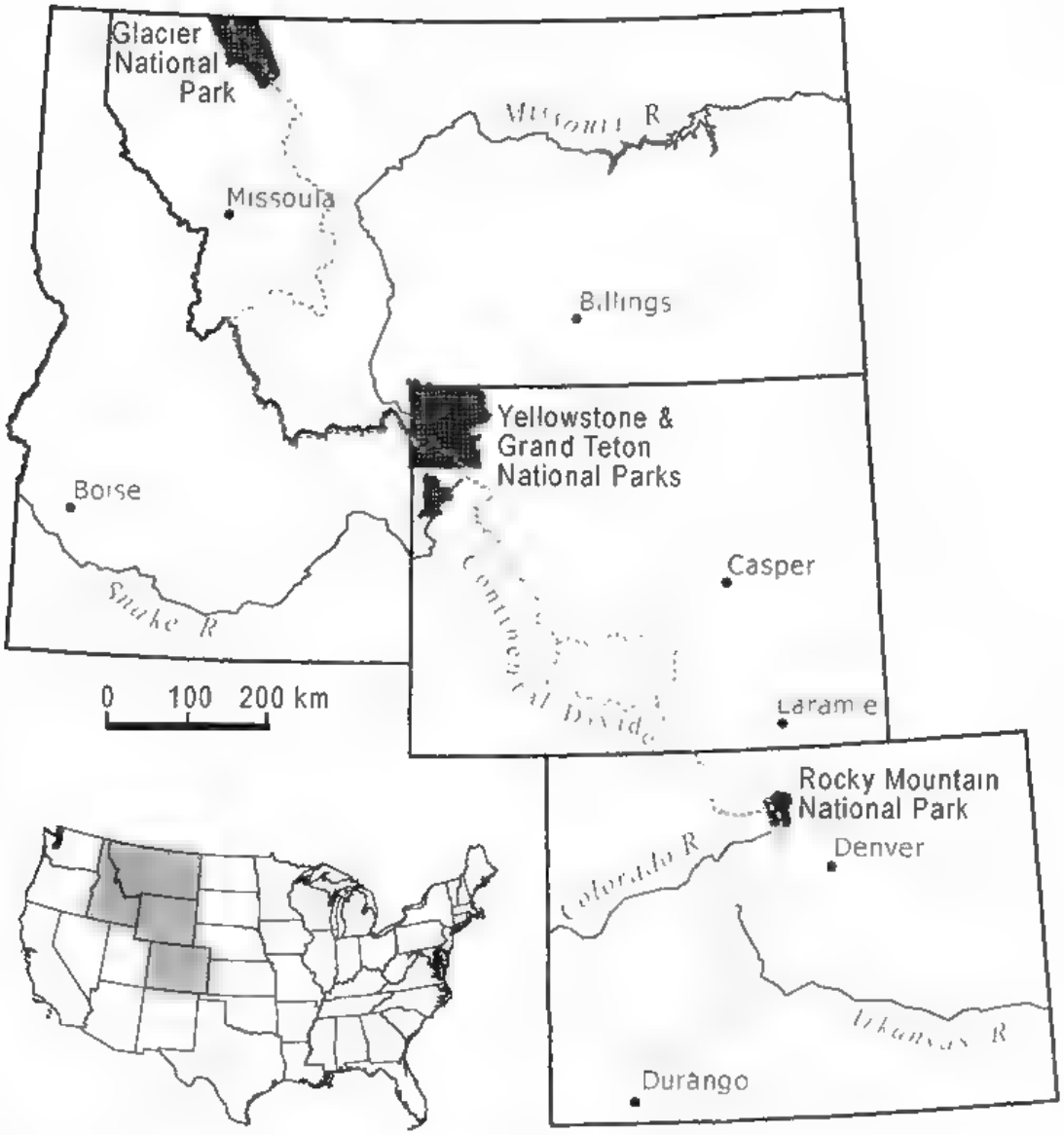


Fig. 1. The three study areas are distributed on the Continental Divide. The scale bar refers to the four states figured and not to the inset United States map.

enough data are collected for reliable analyses. However, failure to detect presence of a species during a visit to a site may not mean that the species is absent. Therefore, we employ multiple visits to sites and recently-developed statistical tools (MACKENZIE et al., 2002, 2003) that incorporate detection probabilities to estimate occupancy.

The ultimate goal of Rocky Mountain ARMI is to conduct these surveys over the long term, in collaboration with the National Park Service. The data and analyses will provide park managers with valuable information, such as whether declines seen so far in the south are

Source : MNHN, Paris

Table 1. – Selected statistics describing the location, climate, and human influences on the three study areas on the Continental Divide. Park areas and use statistics are from ANONYMOUS (2004). Climate data are the averages of National Climate Data Center reporting stations within or at the margins of the parks.

Trait	Glacier	GYE	Rocky Mountain
Size (ha)	410,178	1,033,389	107,577
Central latitude	48°37'N	44°22'N	40°22'N
Central longitude	113°49'W	110°33'W	105°42'W
2003 recreational visits	1,664,046	5,375,068	3,067,256
2003 backcountry overnight stays	22,958	59,036	36,012
Nearby population[1]	851,000	528,000	3,925,000
1961-1990 mean annual temperature (°C)	4.7	2.7	4.2
1961-1990 mean annual precipitation (mm)	614	527	457

[1] United States 2000 Census, sum of county populations where the central coordinate of the county lies within 150 miles (241 km) of the central coordinate of the study area, Canada: 2001 Census, sum of Census Divisions 02 and 03 in Alberta and 01 and 03 in British Columbia (<www.statcan.ca>).

moving north, or whether amphibians that have declined are beginning to recover. Our objectives in this paper are to describe the design of surveys on the transect, report the rates of site occupancy for each species for the first two years of observations, and provide initial estimates of trend in site occupancy for *B. boreas* in Glacier.

METHODS

Our sampling units were a set of hierarchically nested drainage catchments from the USGS Elevation Derivatives for National Applications Project (KOST & KELLY, 2001). Drainages were overlaid onto National Wetlands Inventory (NWI) maps and aggregated to include 10-50 identifiable water bodies, which resulted in 40 catchments in Rocky Mountain and 80 in Glacier. There were 1060 catchments in GYE before aggregation. Because of the large number of catchments in GYE, we did not aggregate them until one was selected for sampling. We selected survey units by drawing systematic random samples in each study area to ensure spatial balance. The goal was to survey every accessible water body in selected catchments at least once, with a subset (Glacier and GYE) or all (Rocky Mountain) sites surveyed twice or more to estimate detection probabilities. Duplicate surveys were achieved by independent surveys on different days or on the same day by biologists working independently or working together at different times. For example, areas with numerous small water bodies were sometimes sampled independently by each biologist. Large sites such as wet meadows or river valleys were sampled by two biologists simultaneously. We used visual encounter surveys (HEYER et al., 1994) to search for all life stages of amphibians (embryo, larva and adult) in accessible portions of the water body, using dip nets to sample areas with

Source : MNHN, Paris

limited visibility, and recorded site (e.g., pond area, extent of emergent vegetation) and sampling covariates (e.g., temperature, date) for all surveys (ADAMS et al., 2005). A site was considered to be occupied only if breeding had occurred there, as indicated by presence of egg masses, larvae, or recently metamorphosed juveniles. Presence of adults not engaged in breeding activity was insufficient to consider a site as occupied.

We estimated detection probabilities and occupancy using the program PRESENCE (MACKENZIE et al., 2002). For this analysis, we used the simplest model, assuming constant probabilities of detection and site occupancy. Descriptions of the methods are available elsewhere (MACKENZIE et al., 2002, 2003; MACKENZIE & BAILEY, 2004).

To provide an early assessment of trends in populations of *B. boreas* in Glacier, we compared changes in numbers of occupied sites in three catchments that have been monitored each year from 1999 to 2002 (restrictions due to extensive fires in Glacier in 2003 prevented access to our sites). However, somewhat differing levels of effort among years made estimation of occupancy problematic, so we computed year-to-year estimates of trend (GREEN, 2003) as trend = $\ln(N_t/N_{t-n})$, where N = the number of occupied sites, t = the current year, and n = a previous year (1 to 3, in this case). Only sites that were sampled in both years of the comparison were included in the calculation of trend. Positive values of trend indicate increases in site occupancy, and negative values indicate decreases.

RESULTS

We surveyed 140 (2002) and 79 (2003) sites in 15 catchments in Rocky Mountain, 183 (2002) and 189 (2003) sites in 17 catchments in GYE, and 325 (2002) and 116 (2003) sites in 17 catchments in Glacier. Species detected included *B. boreas*, wood frog (*R. sylvatica*) and boreal chorus frog (*Pseudacris maculata*) in Rocky Mountain, *B. boreas*, *P. maculata*, tiger salamander (*Ambystoma tigrinum*) and Columbia spotted frog (*R. luteiventris*) in GYE, and *B. boreas*, *R. luteiventris* and long-toed salamander (*A. macrodactylum*) in Glacier. We detected *A. tigrinum* at Rocky Mountain, but sampling was inadequate and we do not report these data here. We also observed *P. maculata*, the Pacific treefrog (*P. regilla*) and the Rocky Mountain tailed frog (*Ascaphus montanus*) in Glacier, but these species either have ranges that terminate near the border of the Park and have been found in less than 10 sites each (the hylids) or occur in habitats that we did not sample in these surveys (*A. montanus* inhabits headwater streams). Our observations of *P. maculata*, however, do represent an addition to the fauna occurring in Glacier (HOSSACK & YALE, 2002).

Detection probabilities were high for all species (tab. 2), with the exception of one anomalous value for *P. maculata*. We are unsure of the cause of this low value, but we consider it to be an outlier. Anurans tended to have higher detection probabilities (mean 0.84, not including *P. maculata* in 2003 at Rocky Mountain) than did salamanders (mean 0.68). Estimated occupancy for each species varied between 0 and 0.57 and decreased from north to south (tab. 2). The highest occupancy observed in Rocky Mountain was 0.10 for *R. sylvatica* in 2003, but this species has a restricted distribution in the Park. In contrast, the only occupancy below 0.10 in Glacier was for *B. boreas* in 2002, and *A. macrodactylum* occurred at

Source : MNHN, Paris

Table 2. – Site occupancy and detection probabilities of amphibians in the three study areas. Dashes indicate parameters that could not be estimated due to sparse data.

Study area / *Species*	2002				2003			
	Occupancy (naïve)	Detection probability	Occupancy (adjusted)	Standard error	Occupancy (naïve)	Detection probability	Occupancy (adjusted)	Standard error
Glacier								
Ambystoma macrodactylum	0.38	0.68	0.53	0.058	0.48	0.75	0.57	0.062
Bufo boreas	0.06	0.90	0.07	0.027	0.12	0.74	0.15	0.041
Rana luteiventris	0.14	0.79	0.17	0.028	0.23	0.92	0.24	0.042
GYE								
Ambystoma tigrinum	0.12	0.64	0.18	0.135	0.16	0.62	0.24	0.048
Bufo boreas	0.05	-	0.05	0.016	0.02	-	0.02	0.0[illegible]
Pseudacris maculata	0.38	0.88	0.43	0.05	0.30	0.94	0.32	0.037
Rana luteiventris	0.20	0.75	0.26	0.051	0.14	0.95	0.14	0.026
Rocky Mountain								
Bufo boreas	0.01	-	-	-	0	-	-	-
Pseudacris maculata	0.02	0.71	0.02	0.013	0.09	0.29	0.18	0.118
Rana sylvatica	0.03	-	0.03	0.032	0.10	0.87	0.10	0.055

less than 50 % of sites. Occurrence of amphibian species in GYE was intermediate between Rocky Mountain and Glacier.

There was no apparent trend in numbers of breeding sites of *B. boreas* in Glacier between 1999 and 2002 (tab. 3) There was no trend between 1999 and 2000, an increase in site occupancy between 2000 and 2001, followed by a decrease in occupancy of the same magnitude between 2001 and 2002 Trend over greater intervals shows less variability Comparing 2001 to 1999, there was only a slight increase in site occupancy, and there was no trend between 1999 and 2002.

Discussion

Results of our surveys in 2002-2003 confirm data from earlier studies that amphibian occurrence is greatly reduced in the southern Rocky Mountains. The cause of the decline of *B. boreas* in Rocky Mountain and elsewhere in the southern Rocky Mountains is thought to be the result of infection by the fungus *Batrachochytrium dendrobatidis* (Muths et al., 2003, Carey et al., 2005) Although *B. dendrobatidis* has been detected in *R. sylvatica* and *P. maculata* in Rocky Mountain (Rittmann et al., 2003, Green & Muths, 2005), we do not know how or whether these species have been affected.

There has been relatively little direct physical alteration of amphibian habitats in any of the parks. However, the high visitor use and the greater size of the nearby human population at Rocky Mountain suggests that it is reasonable to hypothesize that anthropogenic influences have contributed directly or indirectly to the low occupancy of amphibians there. For example, Davidson et al (2002) found an anthropogenic influence (amount of agriculture

Source : MNHN, Paris

Table 3. Annual trends in populations of *B. boreas* in three catchments in Glacier National Park. The highlighted diagonal shows the comparisons between each year (columns) and the previous year (rows). Values above the diagonal show comparisons between sites sampled both in the year of each column and 2 or 3 years before.

Year	Sites surveyed	Sites with breeding	Year-to-year trend		
			2000	2001	2002
1999	12	8	0.00	0.12	0.00
2000	18	12	–	0.22	0.08
2001	28	23	–	–	-0.24
2002	41	28	–	–	–

upwind) was correlated with reduced occurrence of four species of amphibians in California. Adjusted for area, Rocky Mountain receives about five times the visitor use, both in the backcountry and by visitors touring the parks by automobile, than do Glacier or the parks in the GYE (tab. 1). Rocky Mountain is the smallest of the parks in the transect, but has the greatest number of people living nearby and may be less buffered from outside influences. For example, land use change (urbanization and agricultural development) on the Colorado piedmont east of the park has resulted in cooler and wetter summers in Rocky Mountain (STOHLGREN et al., 1998), and increased nitrogen deposition from urban and agricultural sources has altered the diatom communities of Alpine lakes (WOLFE et al., 2001). We can offer no direct evidence that human influences have affected the occurrence of amphibians in Rocky Mountain, but this topic deserves further research.

Differences in climate among study areas could influence the patterns we observed. Rocky Mountain is warmer than the GYE, but receives the least precipitation of the three study areas. Climate and extreme weather events can directly affect amphibian populations (CAREY & ALEXANDER, 2003), and the interactions between climate and anthropogenic influences have received little study.

Whether the levels of habitat occupancy seen in GYE and Glacier represent declines of any species is unknown. It is suspected that *B. boreas* should occur at a greater number of potential breeding sites than has been observed recently (KOCH & PETERSON, 1995, MAXELL et al., 2003). Alternatively, the Rocky Mountains have low diversity of amphibians, and it may be that low species diversity and low habitat occupancy are linked. Unfortunately we lack knowledge of historical habitat occupancy rates for any species. The recent data from Glacier indicate no trend since 1999, but this is still too brief a time series to draw firm conclusions.

The goal of Rocky Mountain ARMI is to collect data over the long term, so that we will be able to draw conclusions about trend for each species within the national parks, and patterns across the region. Longer time series will allow the use of more sophisticated models to estimate detection probabilities and occupancy, including rates of population turnover (MACKENZIE et al., 2003). During the first years of monitoring, the effort in each study area was constrained somewhat by funding, resulting in uneven allocation of effort based on size of study areas. Beginning in 2005, we will use size of the areas in allocating samples, which will

Source : MNHN, Paris

result in increased effort at GYE. Future analyses will also incorporate covariates in the estimation of detection probabilities and site occupancy. Site-specific covariates do not change across sampling occasions within years (e.g., many habitat variables), whereas sampling covariates (e.g , date or weather) may change for each sampling occasion. Both site and sampling covariates can influence detection probabilities, but only site covariates are used to model occupancy probabilities. Analyses of trend also will need to include covariates. For example, annual precipitation may determine the number of available breeding sites. Paradoxically, some species may have higher occupancy in dry years, because there are fewer sites available. The increase in occupancy by *B. boreas* in Glacier in 2001, a year with extremely low winter snowpack, may be an illustration of this phenomenon. We need to be able to distinguish changes in occupancy caused by variation in natural conditions, such as habitat changes due to drought and wildfire or outbreaks of infectious disease, from changes caused by anthropogenic influences, such as introduction of a novel pathogen.

Résumé

Les chercheurs de la Rocky Mountain Region de l'United States Geological Survey's Amphibian Research and Monitoring Initiative ont effectué une etude sur le statut des amphibiens le long d'un transect qui suit la ligne de partage des eaux du Canada au Colorado et qui comprend quatre parcs nationaux. L'étude s'est appuyée sur des inventaires par contact visuel pour déterminer l'occupation des sites, avec des visites multiples à un sous-ensemble de sites pour estimer les probabilités de détection pour chaque espece. Pour chaque espèce, les probabilites de détection ont été généralement élevées (plus de 65 %). Un gradient d'occupation des sites a été mis en évidence, la plupart des espèces étant rares dans le sud et relativement communes dans le nord. Par exemple, *Bufo boreas* est proche de l'extinction dans le Rocky Mountain National Park, présent dans moins de 5 % des sites dans les parcs nationaux de Yellowstone et du Grand Teton au milieu du transect, mais présent dans environ 10 % des sites dans le Glacier National Park. La salamandre *Ambystoma tigrinum* est rare a Rocky Mountain et présente dans moins de 25 % des sites à Yellowstone et au Grand Teton, mais *A. macrodactylum* a été observée dans plus de 50 % des sites à Glacier. Il y a de nombreuses différences entre les parcs, telles que la latitude, le climat, l'abondance des visiteurs et la densité de la population humaine dans la région avoisinante. Le degré auquel ces facteurs ont influencé la distribution et l'abondance actuelles des amphibiens est inconnu et devrait faire l'objet de recherches complémentaires.

Acknowledgments

Surveys were funded by the U S Geological Survey and the National Park Service. We thank the administrative, natural resource management, and ranger staffs of Glacier, Grand Teton, Rocky Mountain, and Yellowstone national parks for their cooperation and assistance.

Source : MNHN, Paris

LITERATURE CITED

ANONYMOUS [National Park Service], 2004 – *Statistical abstract 2003*. Denver, Colorado, National Park Service Social Science Program, Public Use Statistics Office: 1-74.

ADAMS, M. J., HOSSACK, B. R., KNAPP, R. A., CORN, P. S., DIAMOND, S. A., TRENHAM, P. C. & FAGRE, D., 2005 – Distribution patterns of lentic breeding amphibians in relation to ultraviolet radiation exposure in western North America. *Ecosystems*, in press.

BRADFORD, D. F., 2005. – Factors implicated in amphibian population declines in the United States. *In* M. J. LANNOO, (ed.), *Amphibian decline: the conservation status of United States species*, Berkeley, University of California Press: in press.

CAREY, C. & ALEXANDER, M. A., 2003 – Climate change and amphibian declines: is there a link? *Diversity and Distributions*, **9**: 111-121.

CAREY, C., CORN, P. S., JONES, M. S., LIVO, L. J., MUTHS, E. & LOEFFLER, C. W., 2005 – Environmental and life history factors that limit recovery in southern Rocky Mountain populations of boreal toads (*Bufo b. boreas*). *In* M. J. LANNOO, (ed.), *Amphibian decline: the conservation status of United States species*, Berkeley, University of California Press: in press.

CORN, P. S., ADAMS, M. J., BATTAGLIN, W. A., GALLANT, A. L., JAMES, D. L., KNUTSON, M., LANGTIMM, C. A., & SAUER, J. R., 2005 – Amphibian research and monitoring initiative: concepts and implementation. *Scientific Investigations Report*, 2005-5015, Reston, Virginia, US Geological Survey.

CORN, P. S., JENNINGS, M. L. & MUTHS, E., 1997. – Survey and assessment of amphibian populations in Rocky Mountain National Park. *Northwestern Naturalist*, **78**: 34-55.

DAVIDSON, C., SHAFFER, H. B. & JENNINGS, M. R., 2002 – Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. *Conservation Biology*, **16**: 1588-1601.

DROST, C. A. & FELLERS, G. M., 1996. – Collapse of a regional frog fauna in the Yosemite area of the California Sierra Nevada, USA. *Conservation Biology*, **10**: 414-425.

GREEN, D. E. & MUTHS, E., 2005 – Health evaluation of amphibians in and near Rocky Mountain National Park (Colorado, USA). *Alytes*, **22** (3-4): 109-129.

GREEN, D. M., 1997 – Perspectives on amphibian population declines: defining the problem and searching for answers. *In* D. M. GREEN (ed.), *Amphibians in decline: Canadian studies of a global problem. Herpetological Conservation*, St. Louis, Society for the Study of Amphibians and Reptiles, **1**: 291-308.

---- 2003 – The ecology of extinction: population fluctuation and decline in amphibians. *Biological Conservation*, **111**: 331-343.

HALL, R. J. & LANGTIMM, C. A., 2001 – The US national amphibian research and monitoring initiative and the role of protected areas. *George Wright Forum*, **18**: 14-25.

HEYER, W. R., DONNELLY, M. A., MCDIARMID, R. W., HAYEK, L. C. & FOSTER, M. S. (ed.), 1994 – *Measuring and monitoring biological diversity: standard methods for amphibians*. Washington, Smithsonian Institution Press. 1-364.

HOSSACK, B. R. & YALE, K. J., 2002 – Geographic distribution: *Pseudacris maculata*. *Herpetological Review*, **33**: 63.

KNAPP, R. A. & MATTHEWS, K. R., 2000 – Nonnative fish introductions and the decline of the mountain yellow-legged frog from within protected areas. *Conservation Biology*, **14**: 428-438.

KOCH, E. D. & PETERSON, C. R., 1995 – *Amphibians and reptiles of Yellowstone and Grand Teton National Parks*. Salt Lake City, University of Utah Press: 1-188.

KOST, J. R. & KELLY, G. G., 2001 – *Watershed delineation using the National Elevation dataset and semiautomated techniques*. Proceedings, 2001 ESRI International User Conference. Redlands, California, Environmental Systems Research Institute, Inc. [Available at http://gis.esri.com/library/userconf/proc01/professional/papers/pap421/p421.htm]

MACKENZIE, D. I. & BAILEY, L. L., 2004. – Assessing the fit of site occupancy models. *Journal of Agricultural, Biological, and Environmental Statistics*, **9**: 300-318.

Source : MNHN, Paris

MacKenzie, D. I., Nichols, J. D., Hines, J. E., Knutson, M. G. & Franklin, A. B., 2003. - Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. *Ecology*, **84**: 2200-2207.

MacKenzie, D. I., Nichols, J. D., Lachman, G. B., Droegf, S., Royle, J. A. & Langtimm, C. A., 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology*, **83**: 2248-2255.

Marnell, L. F., 1997. Herpetofauna of Glacier National Park. *Northwestern Naturalist*, **78**: 17-33.

Matthews, K. R., Pope, K. L., Preisler, H. K. & Knapp, R. A. 2001. – Effects of nonnative trout on Pacific treefrogs (*Hyla regilla*) in the Sierra Nevada. *Copeia*, **2001**: 1130-1137.

Maxell, B. A., Werner, J. K., Hendricks, P. & Flath D. L., 2003. Herpetology in Montana. *Northwest Fauna*, **5**: 1-135.

Muths, E., Corn, P. S., Pessier, A. P. & Green, D. E., 2003. Evidence for disease-related amphibian decline in Colorado. *Biological Conservation*, **110**: 357-365.

Patla, D. A. & Peterson, C. R., 2001*a*. Status and trends of amphibian populations in the Greater Yellowstone Ecosystem. Pocatello, Idaho State University Division of Biological Sciences, Progress Report.

----- 2001*b*. Summary of GYE-ARMI Activities. Pocatello, Idaho State University Division of Biological Sciences, Progress Report.

Pfeff, R. K., 1988. - Forests of the Rocky Mountains. *In* M. G. Barbour & W. D. Billings (ed.), *North American terrestrial vegetation*, Cambridge, Cambridge University Press: 63-101.

Rittmann, S., Muths, E. & Green, D. E., 2003. *Pseudacris triseriata* (western chorus frog) and *Rana sylvatica* (wood frog). Chytridiomycosis. *Herpetological Review* **34**: 53.

Stohlgren, T. J., Chase, T. N., Pielke, R. A., Sr., Kittel, T. G. F. & Baron, J. S. 1998. Evidence that local land use practices influence regional climate, vegetation, and stream flow patterns in adjacent natural areas. *Global Change Biology*, **4**: 495-504.

Van Kirk, R., Benjamin, L. & Patla, D. A., 2000. *Riparian area assessment and amphibian status in the watersheds of the Greater Yellowstone Ecosystem*. Bozeman, Montana, Greater Yellowstone Coalition, Final report.

Wolfe, A. P., Baron, J. S. & Cornett, R. J., 2001. Anthropogenic nitrogen deposition induces rapid ecological changes in alpine lakes of the Colorado Front Range (USA). *Journal of Paleolimnology*, **25**: 1-7.

Corresponding editor: C. Kenneth Dodd, Jr.

© ISSCA 2005

Source : MNHN, Paris

Evidence of decline for *Bufo boreas* and *Rana luteiventris* in and around the northern Great Basin, western USA

Wendy H. WENTE [1], Michael J. ADAMS & C. A. PEARL

US Geological Survey, Forest and Rangeland Ecosystem Science Center
3200 SW Jefferson Way, Corvallis, Oregon 97219, USA

Revisiting historical sites for patch-associated fauna can provide a relatively fast assessment of species status in a region. Between 2000 and 2003 we repeatedly surveyed historically documented sites for two amphibian species of concern in the northern Great Basin: the western toad (*Bufo boreas*) and the Columbia spotted frog (*Rana luteiventris*). We estimate that *B. boreas* occupies 49.5 % (34.1-65.0 %) of 34 Historical sites and that *R. luteiventris* occupies 52.9 % (43.5-62.3 %) of its 30 Historical sites. *B. boreas* was more likely to be detected at Historical sites that were human-altered whereas *R. luteiventris* was more likely to be detected at sites that had deeper water than other sites in our study. *B. boreas* was detected at three of 41 (7.3 %) Proximal sites (located near Historical sites) whereas *R. luteiventris* was detected at six of 16 (37.5 %) Proximal sites. *B. boreas* was detected at one of 187 Randomly selected sites, and *R. luteiventris* at three sites. Given that the number of Historical sites available for resurveys was small, our results should be interpreted with caution. Moreover, a species can shift its distribution away from Historical sites due to habitat succession or metapopulation dynamics without necessarily declining. We suggest that there is sufficient evidence to conclude that *B. boreas* and *R. luteiventris* have declined in and around the northern Great Basin. Comparatively high occupancy of Proximal Sites by *R. luteiventris* suggests this species may not have declined as much as has *B. boreas* in our study region.

INTRODUCTION

Declines in amphibian populations are a global problem and their causes are often complex (ADAMS, 1999, KRUSECKER et al., 2001). An essential step in understanding the causes of decline and, perhaps, slowing or halting this process is to document the regional extent of declines. Historical site records, such as those stored in museums or documented in the published literature, are a valuable resource for gauging changes in occupancy patterns of fauna in general (RIZNICK et al., 1994) and have often been used to assess the status of

1. Corresponding author: <wwente@hotmail.com>

Source : MNHN, Paris

amphibians (CORN et al., 1989, ROSS et al., 1995, DROST & FELLERS, 1996, FISHER & SHAFFER, 1996; DAVIDSON et al., 2001; SKELLY et al., 2002)

The Pacific Northwest region of the United States has been a focal area of research on amphibian decline; however, broad-scale studies of amphibian species status remain rare in the region. The scope of most research completed in the region has focused on suspected causal agents such as UVB, disease, and introduced species (e.g. BLAUSTEIN et al., 1994*a-b*, ADAMS et al., 2001; PEARL et al., 2004). Declines of several anurans in the west have been attributed to changes in land use patterns, specifically, habitat destruction and the use of agrochemicals (DAVIDSON et al., 2001; DAVIDSON et al., 2002). The western toad (*Bufo boreas*) has experienced dramatic declines in other parts of its range (CORN et al., 1989; ROSS et al., 1995; DROST & FELLERS, 1996, VERTUCCI & CORN, 1996; FISHER & SHAFFER, 1996), but its status is unknown in the northern Great Basin, an arid region in the intermountain northwest of the United States with large public land holdings. Likewise, the Columbia spotted frog (*Rana luteiventris*) is thought to be declining in portions of its range (PATLA, 1997; REASER, 1997) and is a Candidate Species under the US Endangered Species Act (ANONYMOUS, 1997). To determine the status of these amphibians in and around the northern Great Basin, we documented the occupancy of three types of sites by *B. boreas* and *R. luteiventris*: historically occupied sites (Historical sites), sites located near the Historical sites (Proximal sites), and randomly selected sites from across the region (Random sites). We also analyzed how habitat characteristics and certain potential stressors affect amphibian occurrence at Historical sites.

MATERIALS AND METHODS

We developed a list of Historical sites from museum records, published accounts, and field notes of local biologists. Records ranged in date from 1939 to 1999 but any record with an observation prior to the years of this study could be included as a Historical site. Most Historical sites we selected (n = 34 for *B. boreas*, n = 18 for *R. luteiventris*) were located in Oregon east of the Cascade Range but we also included a small number of *R. luteiventris* Historical sites (n = 12) in northeastern Nevada. The overall study area including both Oregon and Nevada ranged from 45°57'35"N, 117°28'27" W in the north to 40°19'16"N, 116°05'07"W in the south, and from 42°41'14"N, 117°01'29" W in the east to 43°54'31"N, 121°45'37" W in the west. Sites ranged in elevation from 406 to 2,256 meters. We gave priority to Historical sites that had records of breeding (i.e., eggs, larvae or newly metamorphosed individuals) but we also included some adult-only sites to increase our sample size. Aside from status at known Historical locations, to determine how rare each species is in the Upper Great Basin habitat of southeastern Oregon, we also surveyed sites in the vicinity of the Historical sites (usually within 25 km). When possible, these Proximal sites were located within the same watershed or were physically similar to the Historical sites. Finally, occurrence data from 187 Randomly selected sites in the southeastern quarter of Oregon were also included in the study. Random sites were drawn using a spatially balanced design (general randomized stratified design with unequal probability sampling, STEVENS & OLSEN, 2004) that provided a widely spaced random selection of fifth field hydrologic units (HUCs). Within the selected HUCs, we

Source : MNHN, Paris

surveyed all accessible ponds and streams located on US Department of the Interior lands. We visited sites indicated on GIS maps developed by the US Bureau of Land Management (BLM) and 7.5 minute US Geological Survey topographic maps as well as sites incidentally encountered in the field (less than 5 % of total number visited).

The region covered by our surveys roughly coincides with, but in some cases exceeds, the northern Great Basin. Some Oregon Historical and Proximal sites were located on the east slope of the Cascades and in the Blue Mountains ecoregion around the margins of the northern Great Basin. Our surveys focused on lands managed by the BLM and therefore excluded higher elevation lands managed by the US Forest Service (USFS) in northern and central Nevada where REASER (1997) has previously documented a decline of *R. luteiventris*.

In 2000-2003, we surveyed sites between April and September when amphibians are most likely to be active. All sites in Oregon (n = 52) were surveyed in more than one year and a few (n = 14) were surveyed more than once during a single field season. Sites in Nevada (n = 12) were surveyed once. We excluded data from sites that were dry. We used a standard visual encounter survey method including wading and dipnetting to detect amphibians (OLSON et al., 1997). At each site, we recorded all amphibian taxa and life history stages as well as 10 site and habitat characteristics. Our protocol called for two workers to slowly search all habitats within one meter of standing water. The workers turned any cover objects by hand and used a long-handled dip net to sweep through open water, aquatic vegetation, or along the substrate. At larger lentic sites only a partial survey was completed. If the historical record included specific locality details about a large site, that portion was searched. Otherwise, we searched areas with appropriate pool and vegetative cover characteristic for amphibian habitat (expert opinion).

STATISTICAL ANALYSIS

Because detection of amphibians at ponds is expected to be less than 100 % (OLSON et al., 1997), we used the program PRESENCE (MACKENZIE et al., 2002) to estimate the proportion of Historical sites that are currently occupied in addition to reporting the proportion of sites where we detected the species (the naïve occupancy rate). PRESENCE is designed to estimate the proportion of sites occupied (Ψ) by a species of interest while incorporating an estimate of species detectability (p). There are two possible explanations of a non-detection result at a surveyed site: either the species was not present at the site or we failed to detect it even though it was present. By visiting a site multiple times, we were able to estimate species detectability and produce an unbiased estimate of occupancy. We treated all searches as if they were conducted during a single season. This violates an assumption of PRESENCE that occupancy does not change during the study. This violation may have caused us to underestimate detectability by treating some true absences as false absences. Underestimating detectability would cause us to overestimate occupancy so any declines may be more severe than our estimates indicate.

PRESENCE also allows for the inclusion of covariate data. We constructed models to estimate occupancy using the response variable Detection along with one survey covariate and nine site covariates that were selected from the larger suite of characteristics collected

Source : MNHN, Paris

Table 1. Variables used in models to describe detection data for *Bufo boreas* and *Rana luteiventris*. Class refers to the variable type (response variable, survey or site covariate) as well as the category it represents (habitat or stressor).

Variable	Class	Description
Detection	response	binomial: presence or non-detection of species at site
Season	survey	binomial: indicates if detection in spring (April-May) differs from summer (June-September)
Record Type	site	binomial: historical evidence of breeding or not
Elevation	site/habitat	continuous: elevation of site in meters
Cover	site/habitat	continuous: per cent emergent vegetation at site averaged across visits
Shallows	site/habitat	continuous: per cent shallows (< 0.5m) at site averaged across visits
Wetland Type	site/habitat	binomial: site was pond/lake/spring or river/stream
Origin	site/habitat	binomial: naturally occurring or human-altered/human-made
Grazing	site/stressor	binomial: evidence of cattle grazing at site or not
Distance from Road	site/stressor	binomial: site was less than or greater than 100 m from nearest road
Fish	site/stressor	binomial: fish of any species detected at site or not

during each Historical site survey. The response variable in all cases was coded 1 if the species was detected during the given survey and 0 if it was not. We limited the suite of covariates to 10 due to our small sample sizes (tab. 1). The survey covariate Season indicated whether the site was surveyed in spring (April-May) or summer (June-September). This was included because we suspected that detectability was lower in the summer than in the spring. One site characteristic, Record Type, indicated whether the site historically had a record of breeding or non-breeding. Besides the survey covariate and Record Type, we included variables that, as indicated by previous work (Hadden & Westbrooke, 1996; Aker, 1998, Vos & Chardon, 1998, Semlitsch, 2000; Pilliod & Peterson, 2001), might be related to amphibian decline. Grazing (whether or not the site was used by cattle), Distance to Road (whether or not the site was less than 100 m from the nearest road) and Fish presence (whether or not fish were detected). We also included variables that are often important to amphibian biology: Elevation, Cover (percentage of the site covered with emergent vegetation), Shallows (percentage of the site with water less than 0.5 m in depth), Origin (whether the site appeared to be natural or if it was human-altered/human-made) and Wetland Type (lentic or lotic). We screened all 10 of the predictor variables for collinearity. If a pair of variables had a Pearson correlation above 0.70, one must be eliminated (Nash & Bradford, 2001); however, none of our variable pairs broke this rule. We also screened for multiple collinearity by building a simple linear regression model with detection as the response variable. In the collinearity diagnostics, a variance inflation factor above 10 for any of the covariates would have indicated a strong linear relationship with one or more of the other covariates and that variable would be dropped (SPSS 9.0, SPSS Inc., Chicago, IL, USA). None of the covariates needed to be dropped. We then built a model predicting occupancy for each species using PRESENCE to conduct a forward stepwise procedure. At each step of the stepwise selection process, we added the variable that resulted in the best model based on the small sample version of

Source : MNHN, Paris

Akaike's Information Criterion (AIC_c). AIC_c is a measure of the information content of a model relative to the number of parameters in the model (BURNHAM & ANDERSON, 2002). We stopped adding covariates when none decreased the AIC_c score by greater than 2 0 following BURNHAM & ANDERSON (2002). Our estimate of site occupancy was based on the model that resulted from this procedure. We report the estimated proportion of sites occupied (y) and a 95 % confidence interval.

As we progressed through the stepwise regression, each model was tested for goodness-of-fit using the Pearson chi-square test statistic and a parametric bootstrap method available in PRESENCE. If a model had failed the goodness-of-fit test (none failed) we would have adjusted our model selection procedures following MACKENZIE & BAILEY (in press).

Finally, we present raw detection data for Proximal and Random sites to provide a more complete depiction of *B. boreas* and *R. luteiventris* status in the region.

RESULTS

Bufo boreas

We detected *B. boreas* at 11 of 34 Historical sites yielding a naive occupancy rate of 32.4 % (fig. 1-2) Using PRESENCE, we estimated the proportion of sites occupied to be 49 5 % (34 1–65.0 %) and the detection probability for a single survey to be 0 3204. Nineteen of our 34 Historical records were pre-1990; we found *B. boreas* at four of these sites (21 1 %) At 15 more recently recorded sites (1990-1999), occupancy was higher (46.7 %). In eastern Oregon, we detected *B. boreas* at three of 41 (7 3 %) Proximal sites and at one of 187 Random sites (0.5 %).

Origin was the only covariate selected using stepwise regression (tab. 2). The odds of *B. boreas* occupying an altered site were 9 2 times greater than for naturally occurring sites. Season did not affect detection probability for this species.

Rana luteiventris

We detected *R. luteiventris* at 12 of 18 (66.7 %) Historical sites in Oregon (fig 3-4) In Nevada, we found *R. luteiventris* at only one of 12 (8 %) Historical sites that had water (six sites were dry) but sites in Nevada were only visited once. Overall, we detected *R. luteiventris* at 43.3 % of Historical sites. Using PRESENCE, we estimated the proportion of sites occupied to be 52 9 % (43.5-62 3 %) and the detection probability for a single survey to be 0.73 Because sites in Nevada were only visited once, we had to assume that detection probabilities were similar in the two states. We found *R. luteiventris* at four of the eight (50 %) Historical sites that were documented before 1990. At 22 more recently recorded sites (1990-1999), the rate of detection was slightly higher (59 1 %) We detected *R. luteiventris* at six of 16 Proximal sites (37.5 %) and three of 187 Random sites (1.6 %).

Source : MNHN, Paris

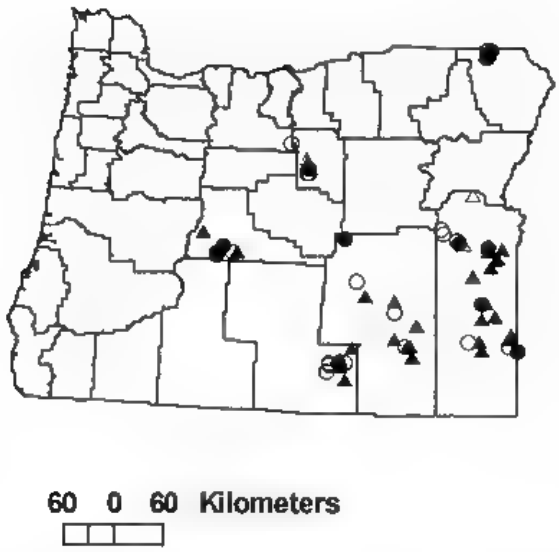


Fig 1 Historical sites surveyed for *Bufo boreas* ($n = 34$) in 2000-2003. Circles indicate Historical sites. Triangles represent Proximal Sites (filled present, empty. *B. boreas* not detected)

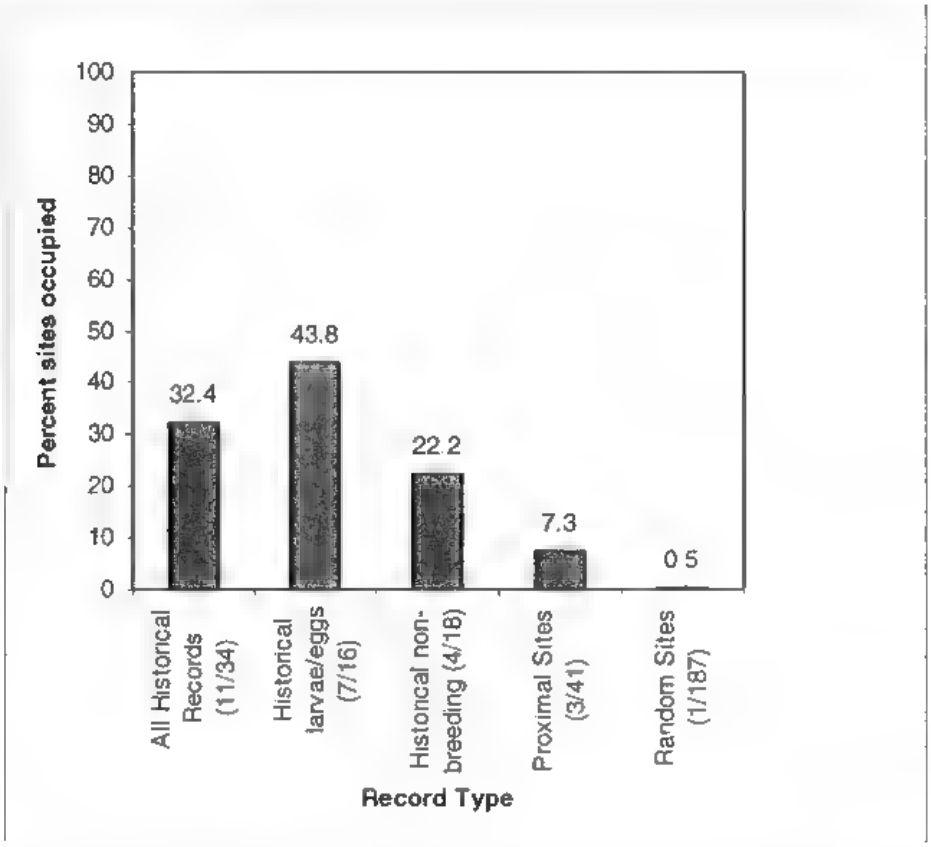


Fig 2 – Occupancy data for *Bufo boreas* at sites in eastern Oregon. In addition to Proximal and Random sites, the data are separated into 3 categories based on evidence of breeding from the Historical record

Source : MNHN, Paris

Table 2. – Models evaluated for best fit to the *Bufo boreas* detection data using the single season model of PRESENCE. *k*: number of parameters; *n*: sample size (number of sites). (.) indicates the null condition where no covariates are included. Site covariates are associated with the occupancy of *B. boreas* at the site (ψ). Survey covariates are associated with *B. boreas* detectability (p). ψ-hat is the estimate of site occupancy for *B. boreas* under the tested model along with standard error. The best model for each step is indicated with bold type.

Model	*k*	*n*	AIC	AIC_c	ψ-hat (SE)
Step 1					
ψ (.) p(.)	2	34	90.4466	90.8337	0.4672 (0.1434)
ψ (.) p(Season)	3	34	91.7882	92.5882	0.4659 (0.1436)
ψ (Record Type) p(.)	3	34	90.984	91.784	0.4606(0.1406)
ψ (Elevation) p(.)	3	34	92.3288	93.1288	0.4714 (0.1473)
ψ (Shallows) p(.)	3	34	91.5037	92.3037	0.4564 (0.1349)
ψ (Cover) p(.)	3	34	91.3205	92.1205	0.4711 (0.1454)
ψ (Wetland Type) p( )	3	34	92.2078	93.0078	0.4615 (0.1399)
ψ (Grazing) p( )	3	34	91.4929	92.2929	0.4760(0.1474)
ψ (Distance from Road) p(.)	3	34	92.411	93.211	0.4690 (0.1448)
ψ (Origin) p(.)	**3**	**34**	**88.3268**	**89.1268**	**0.4953 (0.1542)**
ψ (Fish) p(.)	3	34	92.4284	93.2284	0.49698 (0.1460)

In the stepwise regression analysis, Shallows and Season were the covariates identified as components of the best model describing site occupancy by *R. luteiventris* (tab. 3). For each increase in Shallows of 10 %, the odds of *R. luteiventris* occurring at a site decreased by a factor of 1.8. The detection probability of *R. luteiventris* was higher in spring (April and May).

DISCUSSION

Large-scale, multi-year studies of amphibian status are time and resource intensive. As a result, few have been completed (CORN et al., 1989, FISHER & SHAFFER, 1996; DAVIDSON et al., 2001; SKELLY et al., 2002). Our study was designed to offer a first assessment of species status for *B. boreas* and *R. luteiventris* at sites spread over a large geographic area (eastern Oregon and northeastern Nevada). We found evidence that both *B. boreas* and *R. luteiventris* have declined in eastern Oregon. We failed to detect both species at a large proportion of older Historical records, which might be expected due to a greater likelihood of habitat change at a particular site. We also failed to detect both species at many of the Historical sites known to be occupied within the last 10 years.

Source : MNHN, Paris

Table 3 – Models evaluated for best fit to the *Rana luteiventris* detection data using the single season model of PRESENCE. *k*: number of parameters; *n*: sample size (number of sites). ( ) indicates the null condition where no covariates are included. Site covariates are associated with the occupancy of *R. luteiventris* at the site (ψ). Survey covariates are associated with *R. luteiventris* detectability (p). ψ-hat is the estimate of site occupancy for *R. luteiventris* under the tested model along with standard error. The best model for each step is indicated with bold type.

Model	*k*	*n*	AIC	AIC_c	ψ-hat (SE)
Step 1					
ψ (.) p(.)	2	30	88.1578	88.60224	0.6254 (0.1088)
ψ (.) p(Season)	**3**	**30**	**83.9262**	**84.84928**	**0.5444 (0.1147)**
ψ (Record Type) p(.)	3	30	89.8828	90.80588	0.5107 (0.1100)
ψ (Elevation) p(.)	3	30	88.5946	89.51768	0.5094 (0.1069)
ψ (Shallows) p(.)	3	30	84.6093	85.53238	0.5186 (0.0935)
ψ (Cover) p(.)	3	30	90.1527	91.07578	0.5229 (0.1114)
ψ (Wetland Type) p(.)	3	30	88.2465	89.16958	0.5167 (0.1052)
ψ (Grazing) p( )	3	30	89.0036	89.92668	0.5292 (0.1108)
ψ (Distance from Road) p( )	3	30	85.9227	86.84578	0.5049 (0.0975)
ψ (Origin) p(.)	3	30	89.7491	90.67218	0.5262 (0.1114)
ψ (Fish) p( )	3	30	87.8985	88.82158	0.4967 (0.1012)
Step 2					
ψ (Shallows) p(Season)	**4**	**30**	**80.5017**	**82.1017**	**0.5293 (0.0941)**
ψ (Record Type) p(Season)	4	30	85.8223	87.4223	0.5332 (0.1167)
ψ (Elevation) p(Season)	4	30	84.8601	86.4601	0.5219 (0.1102)
ψ (Cover) p(Season)	4	30	85.9163	87.5163	0.5444 (0.1147)
ψ (Wetland Type) p(Season)	4	30	84.0302	85.6302	0.5364 (0.1078)
ψ (Grazing) p(Season)	4	30	84.7084	86.3084	0.5506 (0.1126)
ψ (Distance from Road) p(Season)	4	30	81.8528	83.4528	0.5205 (0.0996)
ψ (Origin) p(Season)	4	30	85.4464	87.0464	0.5493 (0.1146)
ψ (Fish) p(Season)	4	30	84.1889	85.7889	0.5075 (0.1045)

Although these results seem to demonstrate declines, several factors complicate their interpretation. First, the period of time when our visits were made (2000-2003) coincided with a drought that progressed in severity from moderate to extreme across the region (National Oceanic and Atmospheric Administration National Drought Mitigation Center, unpublished data). This drought was more extreme in the northern Great Basin region of our study and we noted a lower *B. boreas* naive occupancy rate in this region (25 %) than at sites overall (32 %). Individuals in breeding populations of *B. boreas* occasionally skip a year or two of breeding, possibly due to a decrease in foraging opportunities that impact egg production (Olson, 1991). In several amphibian species, skipping of the breeding season has been attributed to

Source : MNHN, Paris

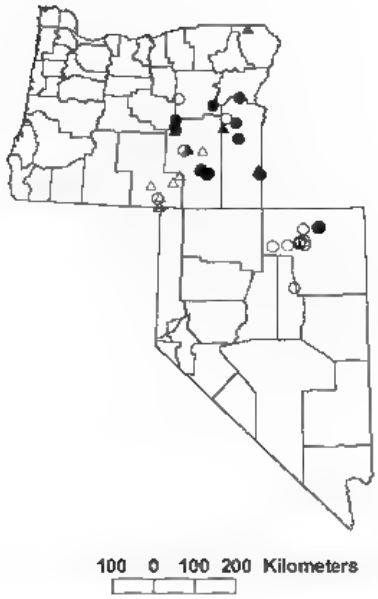


Fig. 3 – Historical sites surveyed for *Rana luteiventris* (n = 30) in 2000-2003. Circles indicate Historical sites. Triangles represent Proximal Sites (filled. present, empty *R luteiventris* not detected).

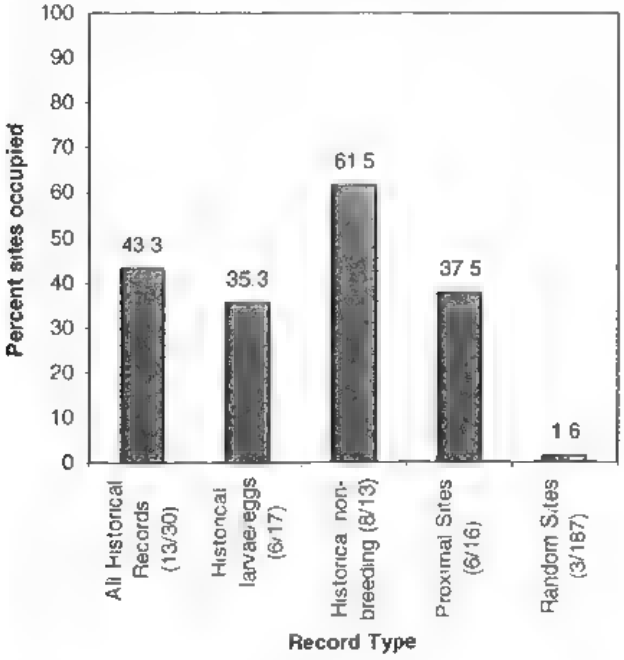


Fig. 4 – Occupancy data for *Rana luteiventris* at sites in eastern Oregon and northeastern Nevada. The larvae/eggs category represents Historical sites where evidence of breeding was found. The non-breeding category represents Historical sites where only adults or juveniles were recorded.

Source : MNHN, Paris

drought (PECHMANN et al., 1991; BABBITT & TANNER, 2000; TRENHAM et al., 2000, MARSH & TRENHAM, 2001). However, the fact that most of our sites were visited for three seasons suggests that *B. boreas* might be permanently absent. Because we excluded Historical sites that were dry from our analysis, our evidence of decline is conservative. Including those sites would have caused our results to indicate a more severe decline.

A second factor that could complicate interpretation of these data is the variation in the type of historical record that we used. Although many of our sites had multiple *B. boreas* records, half were based on adult and/or juvenile records rather than direct evidence of breeding. Record Type was not selected in the final model for either species using a stepwise process so we conclude that the stage of the specimen was not strongly related to the odds of current occurrence.

A final complicating factor is that historical site revisitation studies are inherently biased toward detecting declines rather than expansions (SKELLY et al., 2002). In some cases, a population lost from a historical site may be replaced by a new population due to metapopulation dynamics, successional changes in the suitability of other sites on the landscape, or other processes (SKELLY et al., 1999). We have no way to estimate how often population replacement might have occurred. However, available information suggests that both species have high site fidelity (OLSON, 1991; REASER, 1996; ENGLE, 2001; PILLIOD et al., 2002). Observations of colonization are rare but such events may be episodic. For example, following a large wildfire in Glacier National Park, small numbers of *B. boreas* colonized twelve previously unused breeding sites within two years (Hossack & Corn, unpublished data) and both species can colonize newly constructed ponds (C. Pearl, unpublished data). *B. boreas* extensively colonized new ponds on Mount St. Helens following the 1980 eruption (LOVETT, 2000). The fact that a high proportion of *R. luteiventris* Proximal sites were occupied might suggest that declines in this species are less severe than in *B. boreas* despite similar estimates of occupancy for Historical sites.

Whereas our analysis indicates that some *B. boreas* population losses have occurred, these declines do not appear to be as severe as declines described in other regions in the western USA. For example, CORN et al. (1989) documented *B. boreas* at 10 of 59 (17 %) historical sites in Wyoming and Colorado. DROST & FELLERS (1996) found *B. boreas* at only one of eight (12.5 %) historical sites in and around Yosemite National Park, California. These results can be compared with our naive estimate of 32 % occupancy based on multiple surveys over multiple years. CORN et al. (1989) also visited most sites over multiple years but DROST & FELLERS (1996) only visited sites during one year. The higher occupancy rate in our study may indicate that losses around the Great Basin have not been as severe as those in other parts of *B. boreas*' range. Comparable data on *R. luteiventris* status are available from at least three sources. PATLA (1997) reported a dramatic decline in a single large population at Yellowstone National Park, Wyoming, that occurred between the 1950s and 1990s. In southwestern Idaho, *R. luteiventris* was missing from five of 16 (31 %) known sites in 2001 (ENGLE, 2002). REASER (1997) documented *R. luteiventris* at only 50 % of the historical sites she surveyed in Nevada. The low *R. luteiventris* occupancy rate (1 of 12 wet sites) at our BLM sites in northeastern Nevada suggests declines might be more pronounced in lower-elevation habitats than in the higher elevation sites surveyed by REASER (1997) in Nevada. We recommend return surveys at Nevada sites we were only able to survey once.

Source : MNHN, Paris

Potential causes of decline of *B. boreas* in our region could be related to natural processes of succession or human induced habitat change. Grazing by cattle is one of the most extensive uses of public lands in the western United States (BELSKY et al., 1999) and many reservoirs were built on public lands as an improvement specifically for grazing in the northern Great Basin. Amphibians do use these man-made reservoirs but the specific amphibian habitat use patterns across the region are not known prior to the building of the reservoirs. The fact that our final model showed a tendency for *B. boreas* to occupy human-altered sites at least indicates that cattle reservoirs and other human-altered sites provide some suitable habitat for the persistence of the species.

Rana luteiventris may be experiencing a less severe decline than *B. boreas*. We base this conclusion on the fact that this species was present at 37.5 % of the Proximal sites. This is a high rate of occupancy for sites that were only selected for surveys based on their proximity and similarity to Historical sites. Declines appeared more severe in Nevada but the lack of repeat surveys and Proximal sites limits our ability to interpret these data. The significance of the covariate Shallows is likely related to *R. luteiventris*' need for permanent water. The inclusion of the covariate Season can probably be attributed to the Nevada data because these sites were all visited in the summer and we detected *R. luteiventris* at only one site. *R. luteiventris* in nearby southwestern Idaho populations has been infected with a chytrid fungus and other disease organisms in recent years that might be in part responsible for population declines observed at sites in that state (DREW, 2003). In addition, non-native fish have a strong negative impact on site occupancy by *R. luteiventris* in eastern Washington (AKER, 1998) and Idaho (PILLIOD & PETERSON, 2001).

In summary, our study provides evidence of at least a moderate decline in *B. boreas* and *R. luteiventris* in and around the northern Great Basin. Though none of the variables we examined clearly explained the potential declines of these species the overall patterns we observed warrant further research.

RÉSUMÉ

Le réexamen de sites dont la faune a été étudiée dans le passé (sites historiques) peut fournir relativement rapidement des informations sur le statut des populations animales dans une region. Entre 2000 et 2003 nous avons à plusieurs reprises étudié les populations d'amphibiens de sites historiques. Les deux espèces étudiées, *Bufo boreas* et *Rana luteiventris*, sont menacées dans la région du nord du Grand Bassin. Nous estimons que *B. boreas* occupe 49.5 % (34.1-65.0 %) de 34 sites historiques et *R. luteiventris* 52.9 % (43.5-62.3 %) de 30 sites historiques. *B. boreas* s'est avéré plus présent dans les sites historiques altérés par l'homme, tandis que *R. luteiventris* était plus abondante dans les sites à eau profonde que dans les autres sites étudiés. *B. boreas* a été trouve dans trois sites voisins des sites historiques sur 41 (7.3 %), tandis que *R. luteiventris* a été trouvee dans six sites de ce type sur 16 (37.5 %). *B. boreas* a éte trouvé dans un site sur 187 sélectionnés au hasard; et *R. luteiventris* dans trois d'entre eux. Nos résultats doivent être interprétés avec prudence car le nombre de sites historiques disponibles pour reexamen était faible. De plus, la répartition d'une espèce peut s'eloigner des sites historiques en raison de changements dans les habitats ou de la dynamique de ses metapopu-

Source : MNHN, Paris

lations sans pour autant décliner Nos résultats suggèrent que *B. boreas* et *R. luteiventris* ont décliné dans la partie nord du Grand Bassin et alentours. La relative abondance de *R. luteiventris* dans les sites voisins des sites historiques semble indiquer que cette espèce pourrait ne pas avoir autant décliné que *B. boreas* dans la région d'étude.

Acknowledgments

This study was funded by the US Geological Survey Amphibian Research and Monitoring Initiative (ARMI) and the US Bureau of Land Management D. Jarkowsky did much of the work assembling historical records for Nevada. We are also grateful to C Tait, M. Obradovich, R Demmer and R McNatt of the Bureau of Land Management, M Laws and M Bray of the US Fish and Wildlife Service, W Amy of the US Forest Service, A. Cook of the Nevada Department of Wildlife as well as J. Bowerman for their help with the project Other sources of historical site information included the California Academy of Science Collection, Oregon State University, University of Michigan Museum of Zoology, United States National Museum, University of California Davis, Los Angeles County Museum Herpetological Collection, Marjorie Barrick Museum of Natural History at the University of Nevada Las Vegas, and the Berkeley Museum of Vertebrate Zoology. We thank A. Olsen for his help with statistical design and L Bailey for her advice on the statistical analysis. Work was completed under Oregon Scientific Taking Permit # 116-03, Nevada Scientific Collection Permit # S23390 and Oregon State University Institutional Animal Care and Use Permit LAR-ID#2459.

Literature cited

Anonymous, 1997 *Endangered and Threatened Species: Review of Plant and Animal Taxa, Proposed Rule* Federal Register United States Fish & Wildlife Service Department of the Interior, **62** (182): 50 CFR Part 17

Adams, M J, 1999 Correlated factors in amphibian decline Exotic species and habitat change in western Washington. *J. Wild. Man.*, **63**: 1162-1171.

Adams, M J, Schindler, D. E & Bury, R. B., 2001. Association of amphibians with attenuation of ultraviolet-b radiation in montane ponds. *Oecologia*, **128**: 519-525.

Aker, L M 1998 *The effect of native and non-native fish on amphibian populations in northeast Washington* MS Thesis, Cheney, Eastern Washington University: 1-21.

Babbitt, K J & Tanner, G W, 2000 Use of temporary wetlands by anurans in a hydrologically modified landscape. *Wetlands*, **20**. 313-322.

Belsky, A J, Matzke, A & Uselman, S, 1999 Survey of livestock influences on stream and riparian ecosystems in the western United States. *J. Soil Water Conserv.*, **54**: 419-431.

Blaustein, A R, Hoffman, P, Hokit, D, Kiesecker, J, Walls, S & Hays, J, 1994*a* UV repair and resistance to solar UV-B in amphibian eggs a link to population declines? *Proc. natn. Acad. Sci.*, **91**. 1791-1795

Blaustein, A R, Hokit, D G & O'Hara, R K, 1994*b* Pathogenic fungus contributes to amphibian losses in the Pacific northwest. *Biol. Conserv.*, **67**: 251-254

Burnham, K P & Anderson, D R, 2002 *Model selection and multimodel inference a practical information-theoretic approach* Second edition. New York, Springer

Corn, P S, Stolzenburg, W & Bury, R B, 1989 *Acid precipitation studies in Colorado and Wyoming interim report of surveys of montane amphibians and water chemistry* US Fish & Wildlife Service, Fort Collins, Colorado: 1-57

Davidson, C., Shaffer, H. B. & Jennings, M. R, 2001. – Declines of the California red-legged frog. climate, UV-B, habitat, and pesticides hypotheses. *Ecol. app.*, **11**: 464-479

Source : MNHN, Paris

----- 2002. – Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. *Conserv. Biol.*, **16**: 1588-1601.

DREW, M. L., 2003. – *Laboratory report. Spotted frogs in southwester Idaho. Disease investigation and water quality analysis.* Wildlife Health Laboratory, Idaho Department of Fish & Game, Idaho State Department of Agriculture.

DROST, C. A. & FELLERS, G. M., 1996. – Collapse of a regional frog fauna in the Yosemite area of the California Sierra Nevada, USA. *Conserv. Biol.*, **10**: 414-425.

ENGLE, J. C., 2001. *Population biology and natural history of Columbia spotted frogs (*Rana luteiventris*) in the Owyhee Uplands of southwest Idaho: implications for monitoring and management.* MS Thesis, Boise, Boise State University: 1-66.

----- 2002. *Columbia spotted frog Great Basin population (Owyhee subpopulation) long-term monitoring plan. Year 2001 results.* Idaho Department of Fish & Game Report, Boise, Idaho: 1-64.

FISHER, R. N. & SHAFFER, H. B., 1996. – The decline of amphibians in California's great central valley. *Conserv. Biol.*, **10**: 1387-1397.

HADDEN, S. A. & WESTBROOKE, M. E., 1996. – Habitat relationships of the herpetofauna of remnant buloke woodlands of the Wimmera Plains, Victoria. *Wild. Res.*, **23**: 363-372.

KIESECKER, J. M., BLAUSTEIN, A. R. & MILLER, C. L., 2001. – Potential mechanisms underlying the displacement of native red-legged frogs by introduced bullfrogs. *Ecology*, **82**: 1964-1970.

LOVETT, R. A., 2000. – Mount St. Helens, revisited. *Science*, **288**: 1578-1579.

MACKENZIE, D. I., NICHOLS, J. D., LACHMAN, G. B., DROEGE, S., ROYLE, A. & LANGTIMM, C. A., 2002. – Estimating site occupancy rates when detection probabilities are less than one. *Ecology*, **83**: 2248-2255.

MACKENZIE, D. I. & BAILEY, L. L., in press. – Assessing the fit of site occupancy models. *J. Agri. Biol. Ecol. Stat.*

MARSH, D. M. & TRENHAM, P. C., 2001. – Metapopulation dynamics and amphibian conservation. *Conserv. Biol.*, **15**: 40-49.

NASH, M. S. & BRADFORD, D. F., 2001. – *Parametric and nonparametric logistic regressions for prediction of presence/absence of an amphibian.* United States Environmental Protection Agency, EPA/600/R-01/081, Washington, DC: 1-40.

OLSON, D. H., 1991. – Ecological susceptibility of amphibians to population declines. *In: Symposium on Biodiversity of Northwestern California, Santa Rosa, CA*: 55-62.

OLSON, D. H., LEONARD, W. P. & BURY, R. B., 1997. – *Sampling amphibians in lentic habitats.* First edition. Olympia, Washington, Society for Northwestern Vertebrate Biology: i-x + 1-134.

PATLA, D. A., 1997. – *Changes in a population of spotted frogs in Yellowstone National Park between 1953 and 1995: the effects of habitat modification.* MS Thesis, Pocatello, Idaho State University.

PEARL, C. A., ADAMS, M. J., BURY, R. B. & MCCREARY, B., 2004. – Asymmetrical effect of introduced bullfrogs (*Rana catesbeiana*) on native ranid frogs in Oregon. *Copeia*, **2004**: 11-20.

PECHMANN, J. H. K., SCOTT, D. E., SEMLITSCH, R. D., CALDWELL, J. P., VITT, L. J. & GIBBONS, J. W., 1991. – Declining amphibian populations: the problem of separating human impacts from natural fluctuations. *Science*, **253**: 892-895.

PILLIOD, D. S. & PETERSON, C. R., 2001. – Local and landscape effects of introduced trout on amphibians in historically fishless watersheds. *Ecosystems*, **4**: 322-333.

PILLIOD, D. S., PETERSON, C. R. & RITSON, P. I., 2002. – Seasonal migration of Columbia spotted frogs (*Rana luteiventris*) among complementary resources in a high mountain basin. *Can. J. Zool.*, **80**: 1849-1862.

REASER, J. K., 1996. – *Rana pretiosa* (Spotted frog) vagility. *Herp. Rev.*, **27**: 196-197.

----- 1997. – *Amphibian declines: conservation science and adaptive management.* PhD Thesis, Stanford.

REZNICK, D., BAXTER, R. J. & ENDLER, J., 1994. – Long term studies of tropical stream fish communities: The use of field notes and museum collections to reconstruct communities of the past. *Am. Zool.*, **34**: 452-462.

ROSS, D. A., ESQUE, T. C., FRIDELL, R. A. & HOVINGH, P., 1995. – Historical distribution, current status, and a range extension of *Bufo boreas* in Utah. *Herp. Rev.*, **26**: 187-189.

SEMLITSCH, R. D., 2000. – Principles for management of aquatic-breeding amphibians. *J. Wild. Man.*, **64**: 615-631.

SKELLY, D. K., WERNER, E. E. & CORTWRIGHT, S. A., 1999. – Long-term distributional dynamics of a Michigan amphibian assemblage. *Ecology*, **80**: 2326-2337.

Source : MNHN, Paris

SKELLY, D. K., YUREWICZ, K. L., WERNER, E. E. & RELYEA, R. A., 2002. – Estimating decline and distributional change in amphibians. *Conserv. Biol.*, **17**: 744-751.

STEVENS, D. L. & OLSEN, A. R., 2004. Spatially balanced sampling of natural resources. *J. am. Stat. Assoc.*, **99**: 262-278.

TRENHAM, P. C., SHAFFER, H. B., KOENIG, W. D. & STROMBERG, M. R., 2000. – Life history and demographic variation in the California Tiger Salamander (*Ambystoma californiense*). *Copeia*, **2000**: 365-377.

VERTUCCI, F. A. & CORN, P. S., 1996. Evaluation of episodic acidification and amphibian declines in the Rocky Mountains. *Ecol. appl.*, **6**: 449-457.

VOS, C. C. & CHARDON, J. P., 1998. Effects of habitat fragmentation and road density on the distribution pattern of the moor frog *Rana arvalis*. *J. appl. Ecol.*, **35**: 44-56.

Corresponding editor: C. Kenneth DODD, Jr.

© ISSCA 2005

Source : MNHN, Paris

Health evaluation of amphibians in and near Rocky Mountain National Park (Colorado, USA)

D. Earl GREEN* & Erin MUTHS**

* US Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, USA

** US Geological Survey, Fort Collins Science Center, 2150 Centre Avenue, Building C, Fort Collins, Colorado 80526, USA

We conducted a health survey of amphibians in and adjacent to Rocky Mountain National Park (RMNP) to document current disease presence inside RMNP and identify disease outside RMNP with the potential to spread to the Park's amphibians. Amphibians from five sites within RMNP and seven sites within 60 km of Park boundaries were collected and examined. Necropsies (*n* = 238), virus isolation, bacterial and fungal cultures, and histological examinations were carried out on amphibian egg masses (outside RMNP/within RMNP: 26/22), larvae (30/42), imagos (recently metamorphosed individuals) (0/3) and adults (61/67) of five species. Marked infections by a pathogenic chytrid fungus (chyridiomycosis), *Batrachochytrium dendrobatidis*, were detected in three species (*Bufo boreas*, *Pseudacris maculata* and *Rana sylvatica*) from three of five sites within RMNP and in one of three species (*P. maculata*) from three sites outside RMNP. Of the fully metamorphosed individuals tested (*B. boreas*, *P. maculata* and *R. sylvatica*), chytridiomycosis was found in 60 % (*n* = 3), 46 % (*n* = 37) and 54 % (*n* = 7), respectively. Chytridiomycosis was the principal lethal pathogenic infectious disease detected in three amphibian species within or adjacent to RMNP. Higher fungi were isolated from the cloaca and skin of all five amphibian species. Watermolds (Oomycetes) were isolated from amphibian eggs or skin of all five species. No evidence of *Ranavirus* was found in cultures and histological examinations of 176 and 142 amphibians, respectively. Fifteen genera of bacteria were identified in larval and just metamorphosed amphibians, and a potentially pathogenic lungworm, *Rhabdias* sp, was identified in 61.1 % (*n* = 11) of *B. woodhousii* outside RMNP, but in only 2 (15.4 %) *R. sylvatica* within the Park.

INTRODUCTION

Boreal toads (*Bufo boreas*) currently exist as remnant populations in Rocky Mountain National Park (RMNP) (a roughly rectangular 107,625 hectares park in northern Colorado, USA; elevation range 2,440 to 4,345 m, latitude and longitude at approximate center of park. 40°40'N, 105°60'W) where their historic range was once more extensive (CORN et al., 1997) Recent precipitous declines in two of three populations of boreal toads within RMNP

Source : MNHN, Paris

(MUTHS et al., 2003) have put this toad in danger of local extinction. The third population where toads were observed in the recent past, is now thought to be extirpated. Fortunately, breeding toads have been observed at two new additional sites in the Park since the conclusion of our study (Muths, unpublished data).

Boreal toads and northern leopard frogs (*Rana pipiens*) have declined severely throughout the southern Rocky Mountain region in the last 20 years, and many populations have been extirpated (CORN, 2000; CORN & FOGELMAN, 1984, MUTHS et al., 2003). Recent studies have implicated infections by the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, in the decline of toad populations in RMNP (MUTHS et al., 2003), and by ranaviruses in mass mortality events in tiger salamanders (*Ambystoma tigrinum*) in the western United States (JANCOVICH et al., 1997, DOCHERTY et al., 2003). *Basidiobolus ranarum*, another fungus, was implicated in the decline of Wyoming toads (*Bufo baxteri*) (TAYLOR et al., 1999*a-b*) but the diagnosis has been revised to indicate that *B. dendrobatidis* was the pathogen (CAREY et al., 2003).

The long-term goals of our research were to determine additional threats to boreal toads within RMNP and to determine basic health parameters in amphibians that have lived sympatrically with this species historically. Baseline biomedical information for most amphibian populations is lacking. For example, the prevalence of bacterial pathogens and normal gut and skin flora for most amphibian populations is unknown, although amphibians may harbor *Salmonella* spp. and *Leptospira*-like and chlamydial organisms (TAYLOR et al., 2001; O'SHEA et al., 1990, BERGER et al., 1999; REED et al., 2000). *Ranavirus*, a distinct genus of the family Iridoviridae, has been identified as the causative agent in anuran and urodelan mortality events in adjacent states (JANCOVICH et al., 1997; DOCHERTY et al., 2003), but has not been detected in cultures and tissue sections of amphibians within and adjacent to RMNP.

Our immediate objectives were threefold, namely (1) to develop baseline biomedical standards for amphibians in this area; (2) to determine pathogens present in amphibians in RMNP and surrounding areas, and (3) to examine the potential for spread of diseases from outside RMNP to amphibians in RMNP. Based on previous work (MUTHS et al., 2003, RITTMANN et al., 2003), we expected to find *B. dendrobatidis* and possibly ranavirus. No other lethal amphibian diseases have been documented previously in RMNP.

MATERIALS AND METHODS

Within RMNP, all extant populations of boreal toads were sampled (n = 2 sites). Other sites were selected by (1) current presence of one or more of the three other species extant in RMNP (HAMMERSON, 1999), (2) ease of access; and (3) spatial coverage of the Park (fig. 1, tab. 1). Sites outside the Park were selected by: (1) proximity to RMNP (within 60 km); (2) presence of *B. boreas* (n = 1, Twin Lakes Reservoir), (3) presence of amphibians, and (4) ease of access. We collected data on boreal toads, chorus frogs (*Pseudacris maculata*), tiger salamanders and wood frogs (*Rana sylvatica*) in the Park, and boreal toads, Woodhouse's toads (*Bufo woodhousii*) and chorus frogs outside of the Park. Amphibians were collected from June 2000 through September 2002.

Source : MNHN, Paris

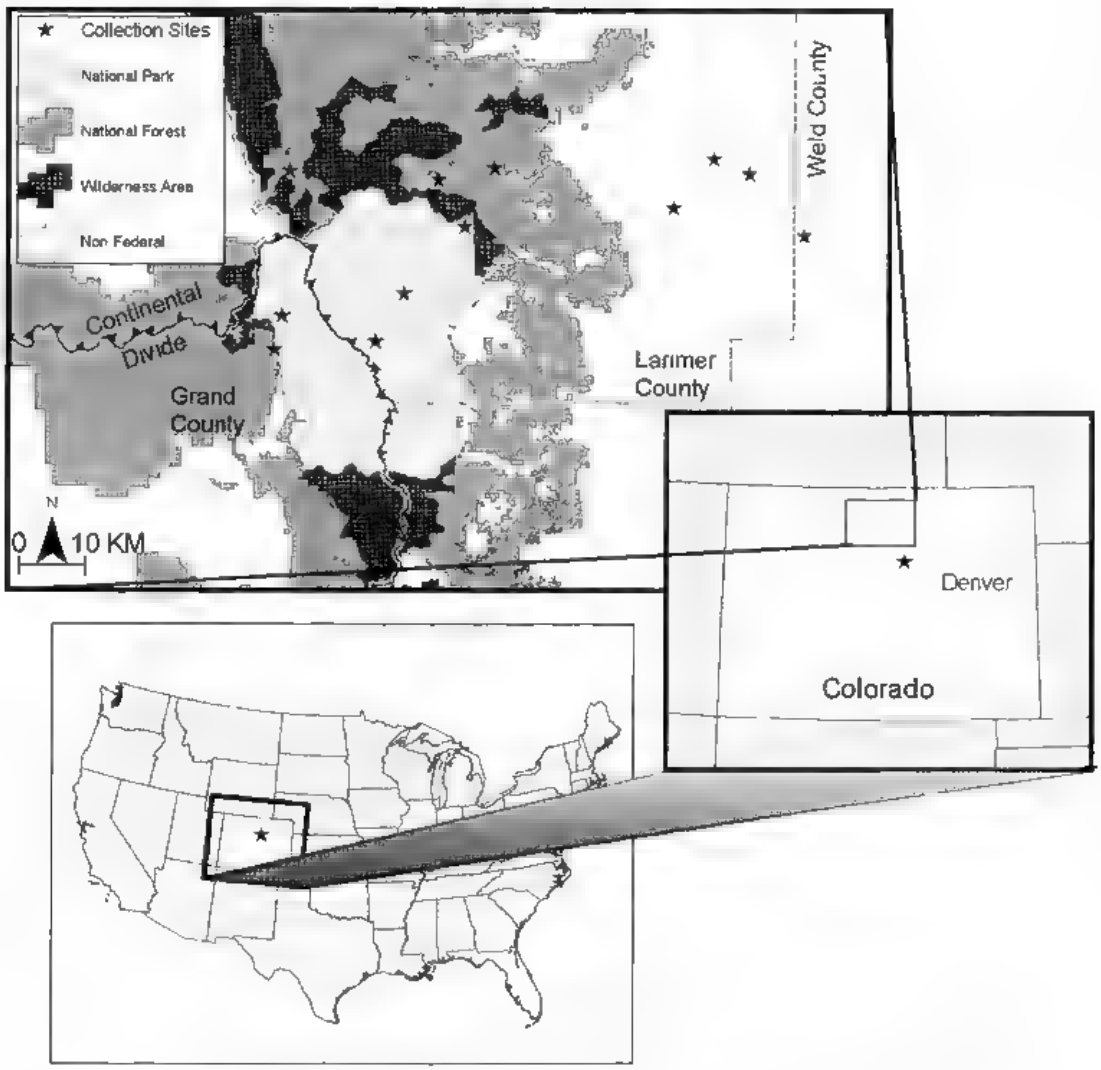


Fig. 1 – Location of Rocky Mountain National Park and surrounding federal and private lands.

FIELD COLLECTION

Adults, imagos (just metamorphosed specimens), larvae and portions of egg masses (approximately 25 eggs per egg mass) were captured by hand or by dipnet. We used disposable latex gloves to handle each animal. All animals were held temporarily and mailed alive in separate containers (toads) or 2-8 animals per container (chorus frogs, wood frogs and tiger salamanders) according to protocols of the United States Geological Survery, National Wildlife Health Center (NWHC) [http://www.nwhc.usgs.gov/research/amph_dc/amph_sop.html]. Adult boreal toads were sampled non-lethally because they are an endangered species in the State of Colorado and have undergone declines statewide (JUNGWIRTH, 2004). Boreal toad populations in the Park are currently monitored using capture-recapture methods

Source : MNHN, Paris

Table 1. Location of sites and distance from RMNP boundary. Negative distances indicate that site is within RMNP. Easting and northing coordinates are North American Datum of 1927 (NAD27) of the Universal Transverse Mercator system, grid zone 13 (UTM13).

Location	County	EASTING	NORTHING	Error (m)	Elevation (m)	Distance (km) (and direction) from boundary
Kettle Tarn	Larimer	455090	4483479	6	2872	-1.21
Spruce Lake	Larimer	441689	4465933	10	2943	-8.10
Horseshoe Park	Larimer	445950	4473115	5	2611	-4.15
Gaskil Ponds	Larimer	426505	4464792	0	2686	-0.47
Timber Creek	Larimer	427573	4469852	0	2715	-1.45
Twin Lake Reservoir	Larimer	451014	4490189	8	2849	3.30 (N)
Lily Pond	Larimer	428649	4491620	8	2969	8.81 (NW)
Horsetooth Reservoir	Larimer	486682	4486065	0	1646	30.32 (E)
Fort Collins	Larimer	492719	4493366	0	1524	37.38 (NE)
Windsor	Weld	506360	4481863	0	1464	48.58 (E)
Pennock Pass	Larimer	459642	4491946	4	2538	7.47 (NE)
Riverbend Ponds	Larimer	498097	4491124	7	1496	42.28 (NE)

with passive integrated transponder (PIT) tags to identify individuals. We used a dilute bath of benzocaine (0.2 % solution, Sigma Chemical Co., Saint Louis, Missouri) to sedate each toad individually When toads were sedated fully (after approximately 5-10 min), they were rinsed in fresh water. The cloaca and oral cavity of adult toads were swabbed twice using Mini-Tip Culturettes (Becton-Dickinson, Sparks, Maryland). Swabs were submitted for virus isolation and bacterial and fungal cultures. Blood (0.5 ml) was collected from anesthetized adult toads (more than 10 g) via heart puncture (Wright, 1995) with single-use, disposable 25 gauge needles and 1 ml tuberculin syringes. Blood was placed immediately into plain hematocrit tubes and sealed with wax. Samples were shipped to NWHC within 48 hours of collection At NWHC, capillary tubes were centrifuged, hematocrit was determined, and serum in capillary tubes was archived (- 70°C). Toads were allowed to recover under observation in the field (30-45 min) In addition to the non-lethal sampling, five boreal toads were found dead and one abnormal live adult toad was collected The live toad was mailed with ice packs and dead toads were promptly fixed in the field by emersion in 10 % formalin.

Laboratory procedures

Necropsy

Amphibians that were dead on arrival at NWHC were necropsied the same day as they were received. Live larvae and just metamorphosed frogs were euthanized in 1:500 solution of MS222 (methanesulfonate salt, Sigma Chemical Co., St Louis, Missouri); adult toads and tiger salamanders were euthanized by applying 2-3 cm of 20 % benzocaine ointment (Orasol gel, Clay-Park Labs Inc, Bronx, New York) to the dorsal midline of head and thorax External and internal examinations were performed using a dissecting microscope equipped with a 35 mm camera.

Source : MNHN, Paris

Hematology

Blood was collected into plain capillary tubes and onto Nobuto blood filter strips (Advantec MFS, Inc., Pleasanton, California) for determination of hematocrit and archiving of sera, respectively, from each metamorphosed amphibian

Virus isolation

Samples of the liver, mesonephros ("kidney") and spleen were pooled for virus cultures and isolations were attempted on fathead minnow cell lines (DOCHERTY et al., 2003).

Bacterial and fungal cultures

Samples of liver, urine, mesonephros, bile, spleen or lung were submitted for aerobic bacterial cultures. A 2 mm × 3 mm segment of cloaca and a 2-4 mm segment of distal toe were submitted for fungal cultures. Tissues and body fluids for routine aerobic bacterial cultures (approximately 1 mm^3) were placed directly into vials of 2 ml tryptic soy broth with glycerine (TSB) and incubated at room temperature (25-27°C). Cultures for *Salmonella* spp. were done in Rappaport-Vassiliadis R10 broth (Becton, Dickinson & Co., Cockeysville, Maryland). Subcultures were performed on 5 % sheep blood agar plates and eosin methylene blue plates. Biochemical identifications of bacterial isolates were performed using the Biolog MicroStation Microbial Identification System (Hayward, California).

Fungal cultures were performed on Sabouraud dextrose agar plates with chloramphenicol and tetracycline (Hardy Diagnostics, Santa Maria, California). Fungal isolates were identified morphologically by features of their hyphae and spores.

Parasitology

Parasites were identified to phylum during necropsies by a pathologist. Some helminths and insects were archived in hot buffered formalin or 70 % ethanol. Identifications to genus were based on external morphology of the live helminths at a dissecting microscope, tissue location in the host and histological features. Representative insects and helminths were identified by parasitologists and aquatic ecologists.

Histology

Portions of ventral skin, digits, heart, liver, lung, spleen, mesonephros, stomach, intestine, pancreas, urinary bladder and gonads were fixed in 10 % buffered neutral formalin, processed routinely, sectioned at 5 microns, and stained with hematoxylin and eosin. Portions of liver, ventral skin, muscle, lung and mesonephros were placed in 1.8 ml cryovials and archived at -70°C at NWHC (Madison, Wisconsin USA).

Source : MNHN, Paris

Table 2. Number and stage of specimens collected from outside (7 sites) and within (6 sites) RMNP. Absent: not detected and not expected to be at site (HAMMERSON, 1999); –: not detected or not collected

Location	Number of (metamorphosed/larvae/eggs) collected				
	Bufo boreas	*Bufo woodhousii*	*Pseudacris maculata*	*Rana sylvatica*	*Ambystoma tigrinum*
Within RMNP					
Kettle Tarn ($n = 1$)	4/0/1	Absent	Absent	Absent	Absent
Spruce Lake ($n = 1$)	11/6/2	Absent	Absent	Absent	Absent
Horseshoe Park ($n = 2$)	Absent	Absent	12/6/6	Absent	6/15/2
Gaskil ($n = 1$)	Absent	Absent	16/0/1	10/0/4	–
Timber Creek ($n = 1$)	Absent	Absent	12/0/2	3/0/2	
Outside RMNP					
Lily Pond	Absent	Absent	15/8/3	0/0/3	Absent
Twin Lakes Reservoir	1/10/0	Absent	19/22/5	Absent	–
Horsetooth Reservoir	Absent	2/0/0	–	Absent	–
Pennock Pass	Absent	Absent	2/0/6	Absent	–
Riverbend Ponds	Absent	3/0/4	–	Absent	–
Fort Collins	Absent	–	6/0/5	Absent	–
Windsor	Absent	13/0/0	–	Absent	–

RESULTS

One-hundred-twenty-one amphibians from 5 sites within the Park and 117 amphibians (5 species) from 7 sites outside of Rocky Mountain National Park were sampled or necropsied (tab. 2).

NECROPSY AND PARASITOLOGY

Ambystoma tigrinum. – Twenty-three individuals were examined from one site within RMNP. Two egg masses were considered normal and free of watermolds. Adult and larval tiger salamanders had spargana (unidentified encysted immature cestodes) within muscles (7 of 15 larvae) and unidentified adult cestodes within gut lumina (7 of 21 larvae and adults). Adult trematodes were present in the urinary bladders of 2 of 6 adult specimens. Encysted metacercariae occurred in the mesonephroi of 14 of 15 larvae. Amputations of extremities (gill and tail tips) were evident in two larvae and one adult but malformations were not found.

Bufo boreas. – Five adults, one imago, six larvae (Gosner stages 40-44) and two egg masses from two sites within RMNP and one site outside the Park were examined. Two adult boreal toad carcasses were desiccated, two were severely autolyzed and one was submitted in formalin. One live adult toad was submitted because of its moribund state at capture and small red blisters were present in its ventral skin. Unidentified adult trematodes were found in

Source : MNHN, Paris

the urinary bladder of one specimen; no other helminths were detected probably because of poor post mortem condition of four carcasses. Deformities were limited to one short hindlimb digit (brachyphalangy) in one toad. One tadpole had mild scoliosis of the tail and all had marked depletion of fat bodies.

Bufo woodhousii – Four partial egg masses and 18 adults from three sites outside RMNP were examined. Egg strings appeared normal and free of watermolds. External abnormalities in adult Woodhouse's toads were a focal ulcer in one tubercle and bilateral hypomelanism of tubercles in a second toad. Internally, one specimen had miliary white hepatic foci and three of 18 toads had mild effusions in the lymphatic sacs. Minute larval nematodes were found in the body cavities of two toads and adult *Rhabdias* sp. (1 to 35 per toad) were found in the lungs of 12 of 18 specimens. One toad had adult trematodes in one lung consistent with *Haematoloechus* sp. Additional helminths were found in the small intestines of nine toads; these included unidentified adult cestodes in the duodenums of nine individuals, unidentified nematodes in the cloacae of six individuals and adult trematodes in the mid-intestine of one individual. Three toads had pale gastric erosions or ulcers.

Pseudacris maculata – Twenty-eight eggmasses (9 within the Park from 3 sites; 19 from outside the Park, tab. 2) were examined. Twelve eggmasses were considered normal; 11 eggmasses contained 1.4 to 83 % moldy eggs and another five contained 11 to 100 % dead, mold free eggs. From some sites, minute unidentified pyriform protozoa were visible in the capsules and vitelline spaces of eggs. Red larval insects (*Ablabesmyia* sp.) were present between eggs of 8 eggmasses within and outside of RMNP. Nineteen of 36 tadpoles were normal but five were dead on arrival. Six larvae had deformities: five cases of domed skull (fig. 2) from one site and one case of forked tail tip. One tadpole had oral saprolegniasis and another had non-specific fraying of lower toothrows and lower jaw sheath. Helminthic parasites were observed in four tadpoles, including pinworms (*Gyrinicola* sp.) in one, renal metacercariae (consistent with *Echinostoma* sp.) in three, and unidentified encysted metacercariae within the body cavities of two.

Eighty-two adult chorus frogs were examined. Externally, three adults showed abnormal molts (dys-ecdysis), one had a single minute red ventral skin ulcer. Five chorus frogs had 1-3 short toes (brachyphalangy) and one had a fractured femur. One frog had unilateral microphthalmia. An unidentified beetle was found in the dorsal lymphatic sac overlying the urostyle of one specimen (fig. 3). Internally, two chorus frogs had herniation of viscera through the abdominal wall into lymphatic sacs. Mildly enlarged livers or spleens occurred in three specimens and one adult male had unilateral atrophy of a testis. Four chorus frogs had adult helminths in the intestine. Encysted renal metacercariae and adult trematodes in the urinary bladder were found in seven specimens.

Rana sylvatica – Ten adults, 3 imagos and nine partial egg masses were examined from three sites, two within and one outside RMNP. All eggs were considered normal and free of watermolds. Two eggmasses had *Ablabesmyia*-like larvae burrowing between eggs. Two wood frogs had *Rhabdias* sp. in their lungs. Two imagos (Gosner stage 46) and one adult from the same site had encysted renal metacercariae consistent with *Echinostoma* sp. Two adults had mildly reddened ventral and digital skin and one had brachyphalangy of two digits. One wood

Source : MNHN, Paris

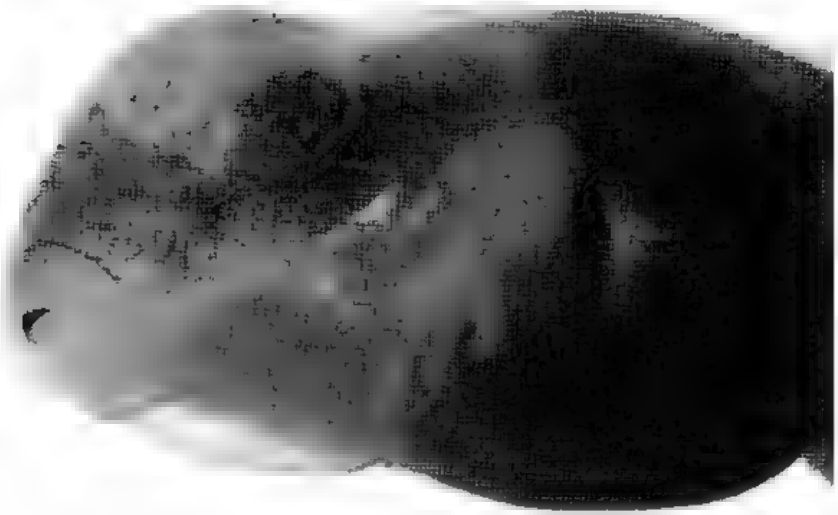


Fig 2 Deformity in larval boreal chorus frog, lateral view of head and body Prominent raised, dome-shaped dorsal skull of unknown etiology occurred in 5 of 36 larvae from one site outside of RMNP Snout-body length of this larva was 9.7 mm.

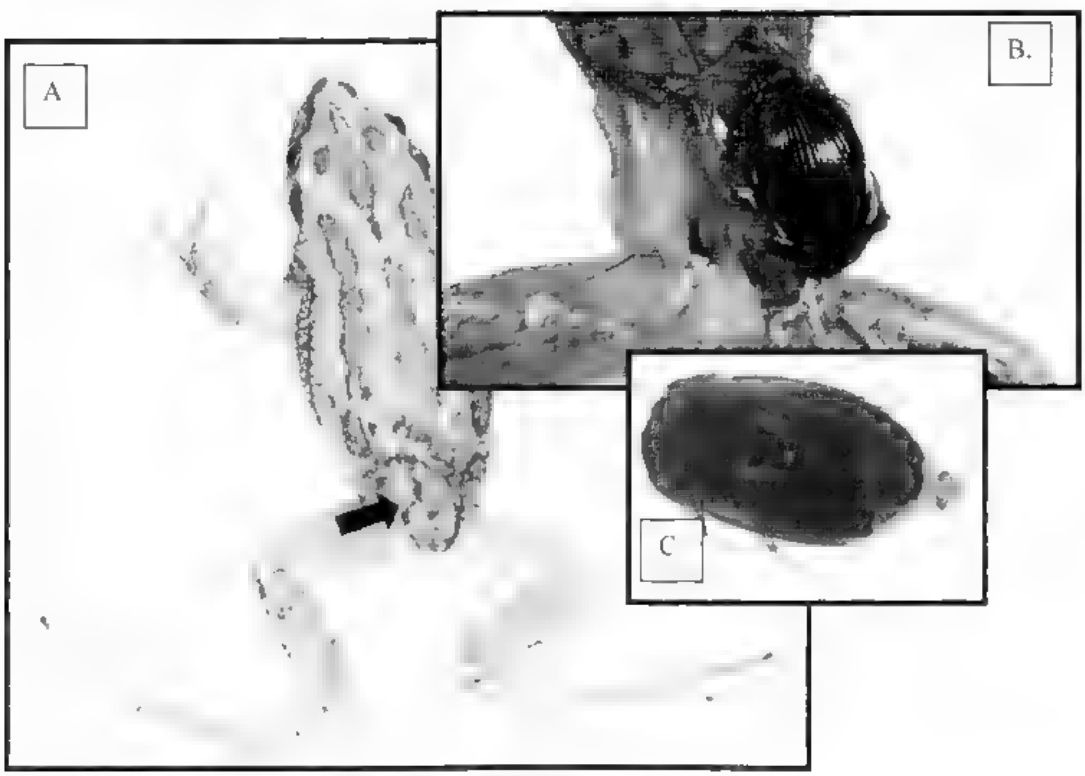


Fig 3 - Parasitism in an adult male boreal chorus frog (A) Dorsal view showing markedly raised and mildly discolored ovoid patch of skin to left of urostyle (arrow) (B) Close-up of dorsal region of urostyle with skin partially reflected to show unidentified adult beetle within lymphatic sac (C) Unidentified beetle removed from lymphatic sac

Source : MNHN, Paris

frog had multiple internal abnormalities suggestive of crushing injury, due possibly to attempted predation or capture. Four of 10 adults had mildly enlarged livers.

HEMATOLOGY

All anesthetized adult boreal toads recovered within 60 min. Eight of 10 anesthetized and heart-punctured boreal toads were recaptured in subsequent years (verified by individual Passive Integrated Transponder [PIT] tag numbers), but recaptured toads were not resampled.

Hematocrits (packed cell volumes, PCV) were determined on 70 adult amphibians and two larval *A. tigrinum*. Mean (number of animals, median; range) PCV for each of the five endemic adult amphibians were: tiger salamanders, 54.6 % (n = 6; 58.6 %; 28.9-65.1 %); boreal toads, 39.9 % (n = 11; 39.7 %, 33.2-44.0 %), Woodhouse's toads, 34.0 % (n = 14; 30.1 %; 17.1-46.4 %), chorus frogs, 32.8 % (n = 34; 32.4 %; 17.7-65.3 %), and wood frogs, 39.8 % (n = 5; 34.4 %; 29.9-54.4 %). Two larval tiger salamanders had PCVs of 34.9 % and 36.8 %. Seven of 34 adult chorus frogs had epidermal chytridiomycosis; these 7 chytrid-positive specimens had a mean PCV of 40.3 % (median: 38.2 %; range: 27.6-65.3 %) while the chytrid-free specimens (n = 27) had a mean PCV of 30.8 % (median: 30.8 %; range: 12.9-49.7 %).

HISTOLOGY

Tissue sections were examined from eggs, embryos, larvae and metamorphosed specimens (n = 179) of all species.

Ambystoma tigrinum. – Minimal to moderate intestinal coccidiosis was detected in eight tiger salamanders. Coccidial oocysts within mucosal epithelial cells contained about 8 sporozoites. An unidentified systemic protozoal infection was detected in the liver, spleen, heart, pancreas or mesonephros of two adult and 3 larval salamanders (fig. 4) from one site in RMNP; protozoal cysts were haemogregarine-like, intracellular, small schizonts 15-25 microns in diameter. Seven larval salamanders had encysted spargana within axial muscles that were 100–700 microns in diameter. Adult cestodes were present in the intestinal lumina of four larval and three adult salamanders. Adult trematodes were present in the urinary bladders of three adult specimens. Nematodes were detected in the intestine of one larva. Encysted metacercariae with encircling granulomatous inflammation were present in the mesonephros of 10 larvae and two adults, presence of small eosinophilic spines in some metacercariae identified the trematodes as *Echinostoma* sp.

Bufo boreas. - Larvae (n = 6) and fully metamorphosed specimens (n = 4) from two sites within RMNP were examined histologically. Two tadpoles had non-specific minimal focal lymphocytic hepatitis and one had minimal bacterial hepatitis. Three of four metamorphosed specimens had mild to severe proliferative (acanthotic and hyperkeratotic) mycotic epidermitis of the ventral and digital skin (fig. 5) typical of chytridiomycosis (BERGER et al. 1998,

Source : MNHN, Paris

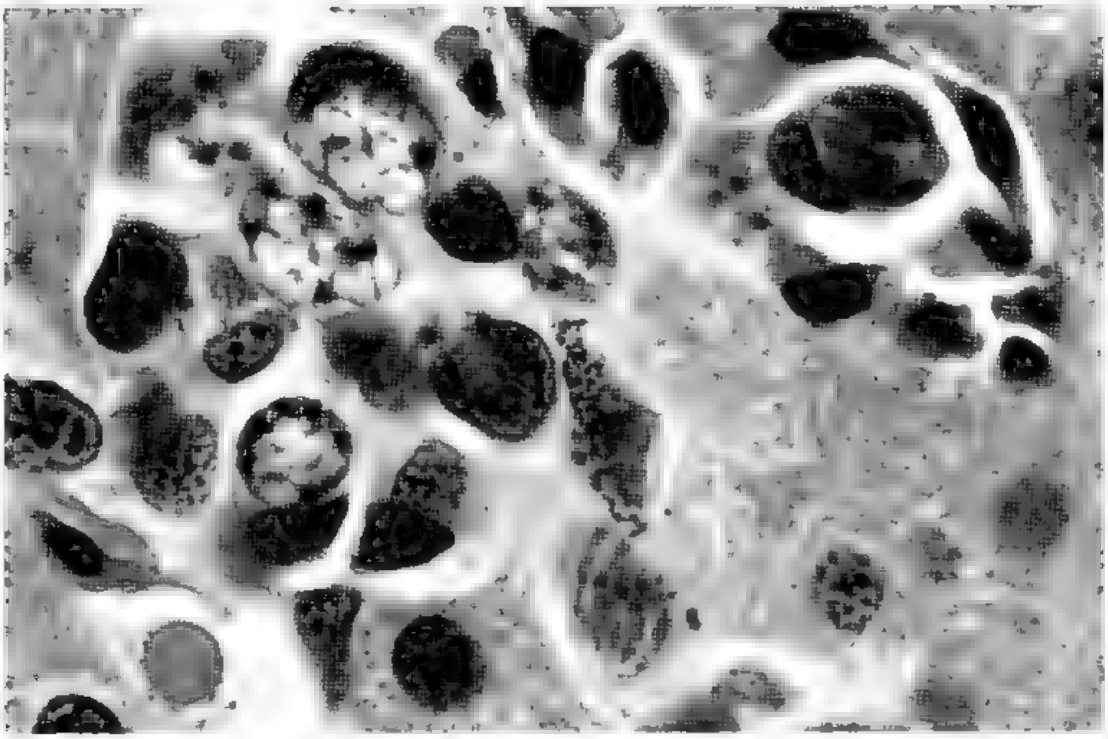


Fig. 4 Protozoiasis of liver of an adult male tiger salamander (24 g, SVL 97 mm) Multiple intracellular protozoal schizonts are present within liver cells or sinusoidal macrophages. Hematoxylin and eosin stain, × 1000

Fig 5 Chytridiomycosis of ventral skin of adult male boreal chorus frog The section shows numerous black, spherical to ovoid chytridial thalli within superficial skin cells, a few thalli have minute thin elongate root-like projections called rhizoids. Warthin-Starry stain, × 1000

Source : MNHN, Paris

MUTHS et al, 2003) Chytrid thalli were not detected in the keratinized structures of the oral disc of the six tadpoles.

Bufo woodhousii Seventeen adult Woodhouse's toads from three sites outside RMNP were examined histologically Chytridiomycosis was not detected in any specimen Histological findings included ova within seminiferous tubules (separate from Bidder's organs) in 3 of 9 adult males, yolk deposition in ova within Bidder's organs of 1 of 9 males, nematodal pneumonia in 9 of 17 specimens consistent with infection by *Rhabdias* sp., mild intestinal coccidiosis in 1 of 17 toads, unidentified adult tapeworms in intestines of 2 of 17 specimens, non-specific minimal acute liver necrosis in 2 of 17 toads, and unilateral cataract of one lens in a female toad. The grossly observed miliary white liver foci in one toad were attributed to granulomatous nodules associated with larval nematodes, whereas the gastric ulcers showed necrosis and sloughing of mucosal cells with no inflammatory cells or organisms.

Pseudacris maculata – Thirty-two larvae and 82 adults (26 female, 56 male) from three sites within and four sites outside RMNP were examined histologically. Three of five tadpoles from one site within RMNP had encysted renal metacercariae consistent with *Echinostoma* sp. Oral saprolegniasis in one tadpole was characterized by acute necrosis of one upper toothrow with clustered watermold filaments. One of 22 larval chorus frog from a site outside RMNP had a marked intracellular protozoal infection of the liver only, the protozoa were not identified. Intestinal pinworms (*Gyrinicola* sp.) were detected in one tadpole.

Adult chorus frogs with chytridiomycosis were found at two of three sites within RMNP and three of four sites outside of Park boundaries. Chytrid fungal infections ranged from minimal to marked in 37 of 82 adult chorus frogs. Within RMNP, 14 of 40 specimens had chytridiomycosis, and outside Park boundaries, 23 of 42 specimens were infected Minimal infections were characterized by chytridial thalli in superficial skin cells with no host reaction, advanced infections showed acanthosis, hyperkeratosis, dysecdysis and large numbers of immature, sporulated and empty thalli. Some infections were accompanied by infiltrates of bacteria into the epidermis and into empty chytrid thalli, no fungal hyphae were seen in the skin

Few additional histological abnormalities were detected in adult chorus frogs. One adult each had a para-hepatic xanthomatous nodule, embedded stomach larval nematodes, eosinophilic cytoplasmic inclusions in duodenal epithelium, acute focal necrotizing mycotic pneumonia, encysted renal metacercariae typical of *Echinostoma* sp., and one intersex frog ("ovotestis")

Rana sylvatica – Thirteen metamorphosed wood frogs from two sites within RMNP were examined histologically Seven (54 %) had minimal to moderate epidermal chytridiomycosis similar in distribution and extent to infections in chorus frogs and boreal toads. Other histological findings were yolk-induced inflammation in the coelom, pneumonia due to *Rhabdias* sp. in two frogs, encysted renal metacercariae due to *Echinostoma* sp. in three specimens, and a displaced (ectopic) nodule of liver tissue in thigh muscles. The four specimens with hepatomegaly had histologically normal livers.

Source : MNHN, Paris

Table 3. – Bacteria cultured from individual amphibians. Numbers indicate numbers of individuals from each in which each bacterium was isolated. AMTI. *Ambystoma tigrinum*; BUBO: *Bufo boreas*; BUWO. *B woodhousii*; PSMA· *Pseudacris maculata*; RASY· *Rana sylvatica*; RMNP: Rocky Mountain National Park; SQ fluid. fluid from lymphatic sacs. Sixty-five organs had no growth; these included 36 livers, 7 spleens, 7 kidneys, 7 eggs, 5 urines, 2 SQ fluids, and one fat body. All isolates are from adults, except· * eggs; ** larvae

Bacteria	Organ	Outside of RMNP				Within RMNP				Total isolates
		BUBO	BUWO	PSMA	RASY	AMTI	BUBO	PSMA	RASY	
Aeromonas encheleia	Cloaca							1		2
	Skin ulcer		1							
Aeromonas hydrophila	Cloaca			1		1**	1	2		6
	Urine		1							
Bacillus spp.	Cloaca						1	1		3
	SQ fluid		1							
Citrobacter freundii	Cloaca		2							2
Enterobacter spp.	Mouth		1				1			9
	Cloaca		1				2			
	Urine		2							
	SQ fluid		2							
Enterococcus spp.	Cloaca							1	1	3
	SQ fluid		1							
Escherichia coli	Cloaca		2							4
	Urine		1							
	SQ fluid		1							
Escherichia vulneris	Liver		1							1
Hafnia alvei	Mouth						1			11
	Cloaca			1			5	1	1	
	SQ fluid								1	
	Liver								1	
Klebsiella spp.	Cloaca						1			1
Pantoea agglomerans	Cloaca						1			1
Pseudomonas spp.	Egg*				3*			2*	5*	33
	Skin ulcer		1							
	Mouth		1			1				
	Cloaca		3	2		1	1			
	Urine		3						1	
	SQ fluid		4							
	Liver		3							
	Skin vesicles						2			
Serratia fonticola	Cloaca						1			2
	SQ fluid								1	
Sphingomonas paucimobilis	Mouth							1		1
Staphylococcus spp.	SQ fluid		1							1
Streptococcus spp	Cloaca		1						1	2
Vagococcus sp	Cloaca					1				1

Bacterial, fungal and watermold cultures

Aerobic bacteriological cultures (oral cavity, cloaca, liver, spleen and kidney) from 24 amphibians (boreal toads, Woodhouse's toads, chorus frogs and tiger salamanders) yielded 18 bacteria of 14 genera, none were known human pathogens (tab. 3) One boreal toad (Spruce Lake), one Woodhouse's toad (outside the Park) and one tiger salamander (Horseshoe Park) tested positive for *Aeromonas hydrophila*. Select cultures of 71 cloacae, intestines and livers of larval and adult amphibians were negative for *Salmonella* spp.

Source : MNHN, Paris

Sixty-two fungal cultures were attempted on cloacae, intestines, mouths and hindlimb digits of 42 larval and metamorphosed amphibians. Special cultures for watermolds (Oomycetes) were attempted on moldy eggs from three egg masses of chorus frogs. *Saprolegnia diclina* was isolated from two dead moldy eggs from two egg masses of chorus frogs, and *Saprolegnia* sp. was isolated in routine fungal cultures of the digits and cloacae of one adult boreal toad, two adult Woodhouse's toads and one adult wood frog. Twenty isolants of *Aspergillus candidus*, *Basidiobolus* spp., *Cladosporium* spp., *Fusarium poae*, *Mucor* sp., *Penicillium* spp. and *Rhizopus* sp. were obtained from the cloacae of 18 larvae and adults of all species. Nineteen isolants of *Aspergillus niger*, *Basidiobolus* spp., *Cladosporium tenuissimum*, *Cladosporium* spp. and *Penicillium* spp. were identified from the digits of 17 adult amphibians of all five endemic amphibians. An unidentified yeast and unidentified fungus of the taxon Zygomycetes were isolated from the cloacae of an adult chorus frog and tiger salamander, respectively. *Basidiobolus* spp. were isolated from 8 of 62 (13 %) amphibians and *Cladosporium* spp. were isolated from 14 of 62 (23 %) larval and adult amphibians. No fungi were isolated from the mouths, cloacae and digits of 33 amphibians.

VIRUS ISOLATION

Cultures were completed on 176 amphibian egg masses, larvae and fully metamorphosed specimens. Organs (lung, liver, spleen and kidney from 164 amphibians of all five species), and 12 sets of oral and cloacal swabs from adult boreal and Woodhouse's toads failed to produce cytopathic effect in fathead minnow cell lines.

DISCUSSION

Whereas the role of emerging infectious diseases in amphibian declines has been examined (e.g.: DASZAK et al., 1999; CAREY, 2000, CAREY et al., 2003), little is known about baseline health of amphibians (but see GLORIOSO et al., 1974, HIRD et al., 1981). Much information published previously on amphibian baseline health and morbidity and mortality events is confounded by recent taxonomic splitting of the presumptive agent of red-leg disease, *Aeromonas hydrophila*, into over 15 species (JOSEPH & CARNAHAN, 1994), discovery of new pathogens (e.g., LONGCORE et al., 1999, JANCOVICH et al., 1997; DOCHERTY et al., 2003) and inadequacies in specimen preservation and length of time between death and necropsy (TAYLOR et al., 2001).

In our study, *Aeromonas* spp. was found in 9.1 %, 33.3 %, 33.3 % and 66.7 % of live free-living boreal toads, Woodhouse's toads, tiger salamanders and chorus frogs, respectively. These data mirror findings by HIRD et al. (1981), who found *Aeromonas* sp. in 32 % (94 of 294) of northern leopard frogs from Minnesota and concluded that presence of this genus of bacterium was not the cause of disease or population declines. The most commonly isolated gut bacteria were *Hafnia alvei*, *Pseudomonas* spp. and *Enterococcus* spp.; these bacteria are considered widespread and innocuous genera in the amphibian digestive tracts and probably reflect water microbiology, invertebrate prey and other environmental features of the amphib-

Source : MNHN, Paris

ian's habitat (WAAIJ et al., 1974). Other bacteria from the amphibian's digestive tracts, such as *Sphingomonas* sp., *Citrobacter* sp. and *Klebsiella* sp. also are common flora of aquatic environments and insects, and likely reflect water quality and prey items (WAAIJ et al., 1974). The mammalian enteric bacterium, *Escherichia coli*, was isolated from three toads captured at one site in an agricultural area near a residence (formerly a farm house) with agricultural fields on two sides. We suggest that coliform bacteria are uncommon in the digestive tracts of amphibians from remote or nearly pristine sites (e.g., RMNP) but may be acquired in amphibians associated with human activities or livestock. Similarly, salmonellae were not isolated from any amphibians in this study; EVERARD et al. (1979) suggested that *Salmonella* spp. are more common in tropical amphibians in close association with humans. Other studies of toads (*Bufo* spp.) in urban and tropical regions found 55.6 %, 36.7 % and 12.7 % to be carriers of salmonellae in Surinam (BOOL & KAMPELMACHER, 1958), India (SHARMA et al., 1977) and eastern Australia (O'SHEA et al., 1990), respectively. TAYLOR et al. (2001) concluded "that most, if not all, amphibians carry one or more *Salmonella* sp.". Our findings refute this statement and provide evidence that toads and other amphibians from temperate zones and high altitudes are seldom carriers of salmonellae.

Basidiobolus spp. are problematic Zygomycetes that are isolated commonly from gut contents of insectivorous amphibians (GUGNANI & OKAFOR, 1980; OKAFOR et al., 1984) but also have been implicated as a primary epidermal pathogen of amphibians (GROFF et al., 1991; TAYLOR et al., 1999*a-b*; TAYLOR & MILLS, 1999). Purported basidiobolomycosis of amphibians is histologically indistinguishable from chytridiomycosis, but basidiobolomycosis in all other vertebrate classes is noteworthy for the presence of fungal hyphae, intense inflammatory cell response, and invasion of non-keratinized tissues (GUGNANI, 1999). Chytridiomycosis of amphibians produces no hyphae, only a slight or no inflammatory cell response and is an intracellular infection of cutaneous keratinized cells only (BERGER et al., 1998). Some published cases of basidiobolomycosis in amphibians are now believed to have been chytridiomycosis (CAREY et al., 2003; MUTHS et al., 2003). Only 1 of 47 amphibians with chytridiomycosis in this study had fungal hyphae in tissue sections, and the hyphae were observed in the lung. Seven of 8 isolants of *Basidiobolus* spp. in this study were from cloacae of adult amphibians. All three isolants of *Basidiobolus* spp. from Woodhouse's toads were cloacal, and all were negative for chytridiomycosis by histology. Fungal cultures of the skin were attempted on 18 frogs (chorus frogs and wood frogs) with histological chytridiomycosis and an additional 21 chytrid-negative amphibians; *Basidiobolus* sp. was isolated from 1 of 39 (2.6 %) skin samples from a chytrid-infected wood frog. We conclude that, in RMNP, *Basidiobolus* spp. are common non-pathogenic fungi in the alimentary tract of amphibians, which supports reports by GUGNANI & OKAFOR (1980) and OKAFOR et al. (1984). The low isolation rate of *Basidiobolus* spp. from amphibian skin suggests that the organism is rare on the epidermis of free-living amphibians, and many skin isolants may be due to fecal contamination of the skin.

A few common and usually innocuous protozoan and helminthic infections were found in some species and populations. Intestinal coccidiosis and cestodiasis were detected in *Ambystoma tigrinum*. Whereas most coccidial protozoa of vertebral wildlife are host specific parasites, life-threatening infections may occur in immature individuals. Because all coccidial infections were considered mild, and because all sympatric chorus frogs ($n = 18$) from the same site were free of coccidia, we conclude that this parasite is not a threat to anuran

Source : MNHN, Paris

populations. However, another unidentified non-enteric systemic protozoal infection was detected in three tiger salamanders from one site within the Park and one adult chorus frog outside of the Park. No morbidity or mortality was associated with this unidentified systemic protozoan infection. Because of low prevalences of these protozoa and absence of infections in sympatric amphibians at each site (which suggests host specificity), we suggest the systemic protozoal infections may be an endemic parasite. The single incidence of a beetle found embedded in the dorsum of a chorus frog is not necessarily a significant finding regarding the parasite load of this species in the Park but is unusual. The frog hosting the beetle was received at NWHC alive, euthanized and dissected immediately indicating that the beetle was not a post-mortem invader.

The only major lethal pathogen associated previously with amphibian mortality events and population declines identified in this study was *Batrachochytrium dendrobatidis*. Other potential pathogens associated with infrequent morbidities and mortalities were intestinal coccidiosis in tiger salamanders, heavy parasitic infections of Woodhouse's toad by the amphibian lungworm *Rhabdias* sp., hepatic or systemic protozoiasis by unidentified protozoa, and saprolegniasis of eggs by *S. diclina*. Virus cultures were negative in all amphibians of all life stages and there was no cultural or histological evidence of bacterial septicemias ("red leg" syndrome).

Chytridiomycosis in boreal toads in RMNP was associated with severe population declines and mortality events in 1998-2000 (MUTHS et al., 2003). This study confirms continued mortality in *Bufo boreas* due to chytridiomycosis within RMNP. In addition, chytridiomycosis was identified in two new amphibian hosts, *Pseudacris maculata* and *Rana sylvatica*, in a total of 44 animals (many more individuals than reported initially by RITTMANN et al., 2003). Histological examinations suggest that the intensity of infections by *B. dendrobatidis* in some amphibians of each species was sufficient to have caused morbidity and mortality. The prevalences of chytridiomycosis in fully metamorphosed specimens of each host species were similar: 60 % in *B. boreas*, 45 % in *P. maculata* and 54 % in *R. sylvatica*. The high prevalences of chytridiomycosis in chorus and wood frogs are worrisome and are equivalent to prevalences in boreal toads, population declines of *B. boreas* have occurred throughout the southern Rocky Mountains (CORN et al., 1997; MUTHS et al., 2003, JUNGWIRTH 2004), but population data for sympatric frogs are unavailable. Monitoring anuran populations in Colorado and Wyoming as well as landscape scale assessments of the number of populations extant in the region are warranted to determine if disease-related declines are occurring. Additionally, experiments to fulfill Koch's postulates using chorus and wood frogs are necessary to verify pathogenicity of *B. dendrobatidis* and determine mortality rates in imagos and adults.

The mechanism of lethality of *B. dendrobatidis* infections in amphibians remains unknown. Our hematological findings support the hypothesis that skin infections by *B. dendrobatidis* disrupt essential functions of the amphibian epidermis. Acanthosis, hyperkeratosis and dyscedysis of the epidermis are associated with advanced *B. dendrobatidis* infections and may impair essential water absorption through the skin and disrupt osmoregulation. The mean hematocrit of infected chorus frogs was 40.3 % whereas non-infected frogs had a mean hematocrit of 30.8 %. There was no difference between the hematocrit values of infected and non-infected animals (ANOVA, $F = 1.73$, $P = 0.20$, $df = 1$). Whereas the mean values were not

Source : MNHN, Paris

statistically different, the possibility remains that impaired osmoregulation (BERGER et al, 1998; DASZAK et al., 1999) and elevated hematocrits (usually indicative of dehydration) occur in some anurans with epidermal chytridiomycosis; additional hematological studies are needed.

Watermold infections (saprolegniasis) in eggs and embryos, some of which were identified as *S. diclina*, affected 25 % (11 of 44) of anuran egg clutches, but in all egg clutches, some live, non-infected embryos were present. Mass mortality of anuran eggs in the Cascade Mountains has been associated previously only with *Saprolegnia ferax* (KIESECKER & BLAUSTEIN, 1995, 1997), but whether *S. diclina* is a primary pathogen, a secondary invader of abnormal eggs, or a saprobe on infertile eggs or eggs killed by other agents could not be determined in this study.

Lungworms of two taxa (*Rhabdias* sp. and *Haematoloechus* sp.) and intestinal coccidiosis were found in *Bufo woodhousii*. Immature, infective stages of the lungworm *Rhabdias* sp. have killed experimental juvenile *Bufo marinus* (WILLIAMS, 1960). *Rhabdias* spp. have a direct life cycle (i.e., without intermediate hosts) and can infect a range of amphibian hosts (FLYNN, 1973), suggesting that this lungworm may infect amphibians in RMNP in situations of crowding, fecal contamination or interspecies contact accompanied by appropriate temperatures and humidity.

Amphibian diversity in RMNP is naturally depauperate, including only five amphibian species (HAMMERSON, 1999). Of these, *Rana pipiens* has been extirpated recently (CORN et al., 1997), *B. boreas* has declined precipitously, and populations of *R. sylvatica* occur only on the west side of the continental divide in RMNP. The latter populations are part of a relictual (meta)population in Colorado and Wyoming that is isolated from other populations in North America (HAMMERSON, 1999). This situation (small, isolated, relict populations) leaves *R. sylvatica* at risk for disease-related extirpation with the subsequent potential loss of genetic diversity.

Given the state of the existing boreal toad populations in RMNP (small isolated populations, continued declines associated with chytridiomycosis), the arrival of another infectious disease could be disastrous. There is potential for natural immigration of healthy (or unhealthy) animals into RMNP, but it is limited. Twin Lakes Reservoir is less than 8 km north of the nearest *B. boreas* breeding site on the north edge of RMNP, and *B. boreas* and *P. maculata* currently are found there. Based on habitat and elevation, boreal toads are expected to be present at Pennock Pass (2552 m), a site less than 10 km north of the Park boundary, but have not been documented there. Lily Pond is less than 9 km northeast of the Park boundary but more than 20 km from the nearest *B. boreas* breeding sites on the north edge of RMNP. *B. boreas* was found at Lily Pond historically (late 1960's) (A. Spencer and P. S. Corn, personal communication) and *P. maculata* and *R. sylvatica* currently reside there. Specimens are not available to test for disease, so it remains unknown why boreal toads disappeared from this site. However, 6 of 15 (40 %) adult chorus frogs captured at Lily Pond in 2001 and 2002 had chytridiomycosis.

There is patchy, appropriate habitat between the locations discussed above and the *B. boreas* sites in RMNP (Arapahoe Roosevelt National Forest Service, Comanche Peak Wilderness Area and National Park). A putative migration route into the Park would include significant elevation gain to the top of Stormy Peaks pass (764 m, Twin Lakes Reservoir;

Source : MNHN, Paris

652 m, Lily Pond, and 1031 m, Pennock Pass), including several kilometers of high alpine habitat (over 3500 m). Boreal toads have been observed in multiple years at elevations of 3383 m (Lake Husted) (CORN et al., 1997) and are capable of moving over substantial distances. We have documented individual toads moving 5 km between breeding sites (over multiple years) (Muths & Corn, unpublished data) such that it would not be surprising to find toads moving from Twin Lakes Reservoir into the northeast portion of RMNP where populations of *B. boreas* currently are found. Migration out of the Park into surrounding areas would be equally likely

Chytridiomycosis has been detected in all four anuran species within and adjacent to RMNP *B. boreas* has suffered severe state-wide declines with chytridiomycosis playing a significant role. Whereas the status of populations of chorus and wood frogs are largely unknown, the prevalence of chytridiomycosis (45 % and 54 %, respectively) is high and equivalent to its prevalence in declining boreal toads in this region (Green, personal observation). The cause of the extirpation of *R. pipiens* in RMNP remains unexplained, although chytridiomycosis was detected in museum specimens that were captured less than 100 km from RMNP in the 1970's (CAREY et al., 2003).

Diseases in *B. woodhousii* were of special interest because chytridiomycosis and other diseases have been reported in several species of *Bufo* in the western United States in recent years (KIESECKER and BLAUSTEIN, 1997, TAYLOR et al, 1999*a*, GREEN & KAGARISE SHERMAN, 2001) *B. woodhousii* occurs only at the periphery of RMNP and although Woodhouse's and boreal toads are thought to be allopatric in Colorado, potential for sympatry exists in Archuleta County (HAMMERSON, 1999) If a change in climate occurs (e.g., prolonged drought), boreal or Woodhouse's toads may begin to use habitat below or above previous elevational ranges. Lungworms of two taxa, in particular *Rhabdias* sp., and intestinal coccidiosis were found in *B. woodhousii*. If the toads become sympatric, *Rhabdias* sp. and coccidial parasites could be transmitted directly to *B. boreas*

The etiologies of musculo-skeletal deformities and gonadal deformities in anurans were not determined Individuals with displaced or ectopic ova within seminiferous tubules were diagnosed as intersexes (ovotestes) in 3 male Woodhouse's toads and one chorus frog; similar abnormal gonads have been reported in *Acris crepitans* in Illinois (REEDER et al., 1998) Abnormal testes may be due to environmental contaminants (e.g., estrogen-mimicking chemicals), or they may be variations of normal anatomy and development. However, one adult male *B. woodhousii* from a partially urbanized site (outside of the Park) had a mild accumulation of yolk (vitellogenin) in ova of Bidder's organs; the production of yolk in a male toad is considered evidence of recent exposure to an estrogen-mimicking chemical (PALMER & SELCER, 1996). The etiology and precise nature of the domed skulls in one group of larval chorus frogs was not determined; additional studies are in progress.

CONCLUSION

Although our study failed to detect any novel diseases associated with high mortality rates (GREEN et al., 2002) in amphibian populations outside the park, there are potential

Source : MNHN, Paris

routes into the park if such diseases are detected in the future. RMNP is surrounded almost completely by National Forest and Wilderness Areas which include appropriate habitat for amphibians. Whereas surrounding public lands may be useful habitats facilitating immigration and dispersal, these areas also could facilitate the transmission of disease into the park since chytridiomycosis has been found in frogs at 3 of 7 sites outside the park. Back country use by sportsmen and domestic animals, including outfitters with pack animals, hikers with dogs and sportsmen using live bait for fishing, could increase the potential of mechanical transmission of pathogens, especially at water sources which may be breeding areas for amphibians. Although the RMNP specifically bans dogs and limits where pack animals can be tethered and on what trails they can use, the Forest Service does not have the same restrictions. None of these potential vectors are proven methods of transmission of amphibian pathogens or are linked directly to the decline of amphibians, the mode of transmission of most major amphibian pathogens remains unknown (Green et al., 2002; Carey et al., 2003). However, anthropogenic movement of nonindigenous species and pathogens are well documented, and at least one major amphibian disease (chytridiomycosis) may be linked to human-mediated transmission (Morehouse et al., 2003).

Of the diseases that have impacts on amphibian populations (chytrid fungus, ranaviruses, and a new mesomycetozoa-like organism, Green et al., 2002), only *B. dendrobatidis* was found within and outside RMNP. Ranaviruses and mesomycetozoan-like organisms were not found in any amphibians in this study. We suggest that the principal hazard to anuran populations within and adjacent to RMNP is chytridiomycosis and that chytridiomycosis is the only major lethal infectious disease of multiple species of amphibians in and around RMNP. Our data are preliminary and limited by sample size and numbers of sites, although amphibians were collected during the months of peak disease activity (Green et al., 2002).

The biomedical data presented here provide information on common bacteria and fungi of free-living amphibians, disease characteristics in populations of amphibians found at higher elevations, and a baseline from which to continue to monitor amphibian health and population declines in the Rocky Mountains.

Résumé

Nous avons etudie les caracteristiques sanitaires des amphibiens au sein du Rocky Mountain National Park et aux alentours pour etablir la présence actuelle de maladies dans le parc et en dehors de celui-ci mais risquant de contaminer les amphibiens du parc. Des amphibiens ont été recoltes et examinés dans cinq sites du parc et sept sites a moins de 60 km des limites de celui-ci. Nous avons effectué des autopsies (n = 238), des isolements de virus, des cultures bacteriennes et fongiques, et des examens histologiques sur des masses d'œufs d'amphibiens (hors du parc/dans le parc: 26/22), des larves (30/42), des imagos (individus recemment metamorphoses) (0/3) et des adultes (61/67) de cinq espèces. Des infections importantes par le champignon chytride pathogène *Batrachochytrium dendrobatidis* (chytridiomycose) ont été detectées chez trois espèces (*Bufo boreas*, *Pseudacris maculata* et *Rana sylvatica*) de trois des cinq sites au sein du par et une des trois espèces (*P. maculata*) de trois sites exterieurs au parc. Chez les animaux metamorphosés des trois espèces (*B. boreas*, *P.*

Source : MNHN, Paris

maculata and *R. sylvatica*), la chytridiomycose a été trouvée respectivement chez 60 % ($n = 3$), 46 % ($n = 37$) et 54 % ($n = 7$) des individus examinés. La chytridiomycose était la maladie infectieuse létale principale détectée chez les trois espèces d'amphibiens au sein du parc ou près de celui-ci. Des champignons supérieurs ont été isolés à partir du cloaque et de la peau des cinq especes d'amphibiens. Des Oomycètes ont été isolés à partir d'œufs ou de la peau des cinq espèces. Aucun *Ranavirus* n'a été trouvé dans les cultures ni par l'examen histologique de 176 et 142 amphibiens des deux provenances. Quinze genres de bactéries ont été identifiés chez des amphibiens larvaires et métamorphosés, et un nématode parasite potentiellement pathogène, *Rhabdias* sp, a été trouvé chez 61.1 % ($n = 11$) des *B. woodhousii* en dehors du parc, mais seulement chez 2 (15.4 %) *R. sylvatica* au sein du parc.

ACKNOWLEDGMENTS

We thank Rocky Mountain National Park and the Department of Interior's Amphibian Research and Monitoring Initiative for funding this project. We are indebted to S. Rittmann for an extraordinary amount of field- and lab-work and care in animal collection and transport. Thanks to J. & S. Chamberlain and to M. Brannon and W. Wolff for access to their property. Thanks also to D. Docherty, R. Long and B. Berlowski (NWHC) for extensive assistance with cultures. We also thank two anonymous reviewers for constructive comments that improved the manuscript.

LITERATURE CITED

BERGER, L., SPEARE, R., DASZAK, P., GREEN, D. E., CUNNINGHAM, A. A., GOGGIN, C. L., SLOCOMBE, R., RAGAN, M. A., HYATT, A. D., MCDONALD, K. R., HINES, H. B., LIPS, K. R., MARANTELLI, G. & PARKES, H., 1998. – Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. *Proc. natl. Acad. Sci. USA*, **95**: 9031-9036.

BERGER, L., VOLP, K., MATTHEWS, S., SPEARE, R. & TIMMS, P., 1999. – *Chlamydia pneumoniae* in a free-ranging giant barred frog (*Mixophyes iteratus*) from Australia. *J. clinical Microbiol.*, **37** (7): 2378-2380.

BOOL, P. H. & KAMPELMACHER, E. H., 1958. – Some data on the occurrence of *Salmonella* in animals in Surinam. *Antonie van Leeuwenhoek*, **24**: 76-80.

CAREY, C., 2000. – Infectious disease and worldwide declines of amphibian populations, with comments on emerging diseases in coral reef organisms and in humans. *Environ. Health Persp.*, **108**, suppl. 1: 143-150.

CAREY, C., BRADFORD, D. F., BRUNNER, J. L., COLLINS, J. P., DAVIDSON, E. W., LONGCORE, J. E., OLELLET, M., PESSIER, A. P. & SCHOCK, D., 2003. – Chapter 6. Biotic factors in amphibian population declines. *In*: G. LINDER, S. K. KRIEST & D. W. SPARLING (ed.), *Amphibian decline: an integrated analysis of multiple stressor effects*, Pensacola, Florida, SETAC Press: 153-208.

CORN, P. S., 2000. – Amphibian declines: review of some current hypotheses. *In*: D. W. SPARLING, G. LINDER & C. A. BISHOP (ed.), *Ecotoxicology of amphibians and reptiles*, Pensacola, Florida: SETAC Press, 663-696.

CORN, P. S. & FOGLEMAN, J. C., 1984. – Extinction of montane populations of the northern leopard frog (*Rana pipiens*) in Colorado. *J. Herp.*, **18** (2): 147-152.

CORN, P. S., JENNINGS, M. L. & MUTHS, E., 1997. – Survey and assessment of amphibian populations in Rocky Mountain National Park. *Northwest Nat.*, **78** (1): 34-55.

Source : MNHN, Paris

DASZAK, P., BERGER, L., CUNNINGHAM, A. A., HYATT, A. D., GREEN, D. E. & SPEARE, R., 1999. Emerging infectious diseases and amphibian population declines. *Emerging infectious Diseases*, **5** (6): 735-748.

DOCHERTY, D. E., METEYER, C. U., WANG, J., MAO, J., CASE, S. T. & CHINCHAR, V. G., 2003. – Diagnostic and molecular evaluation of three iridovirus-associated salamander mortality events. *J. Wildlife Diseases*, **39** (3): 556-566.

EVERARD, C. O. R., TOTA, B., BASSETT, D. & ALI, C., 1979. – *Salmonella* in wildlife from Trinidad and Grenada, W.I. *J. Wildlife Diseases*, **15** (2): 213-219.

FLYNN, R. J., 1973. *Parasites of laboratory animals*. Ames, Iowa, USA, Iowa State University Press. 505-642.

GLORIOSO, J. C., AMBORSKI, R. L., AMBORSKI, G. F. & CULLEY, D. C., 1974. Microbiological studies on septicemic bullfrogs (*Rana catesbeiana*). *Am. J. vet. Res.*, **35** (9): 1241-1245.

GREEN, D. E., CONVERSE, K. A. & SCHRADER, A. K. 2002. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996-2001. *Ann. New York Acad. Sci.*, **969**: 323-339.

GROFF J. M., MUGHANNAM, A., MCDOWELL, T. S., WONG, A., DYKSTRA, M. J., FRYE, F. L. & HEDRICK, R. P., 1991. An epizootic of cutaneous zygomycosis in cultured dwarf African clawed frogs (*Hymenochirus curtipes*) due to *Basidiobolus ranarum*. *J. med. vet. Mycol.*, **29**: 215-23.

GUGNANI, H. C., 1999. - A review of zygomycosis due to *Basidiobolus ranarum*. *Eur. J. Epidemiol.*, **15**: 923-929.

GUGNANI, H. C. & OKAFOR, J. I., 1980. Mycotic flora of the intestine and other internal organs of certain reptiles and amphibians with special reference to characterization of *Basidiobolus* isolates. *Mykosen*, **23**: 26-268.

HAMMERSON, G. A., 1999. – *Amphibians and reptiles in Colorado*. 2nd edition. Boulder, University Press of Colorado and Colorado Division of Wildlife: i-xxii + 1- 484.

HIRD, D. W., DIESCH, S. L., MCKINNELL, R. G., GORHAM, E., MARTIN, F. B., KURTZ, S. W. & DUBROVOLNY, C., 1981. - *Aeromonas hydrophila* in wild-caught frogs and tadpoles (*Rana pipiens*) in Minnesota. *Lab. Anim. Sci.*, **31**: 166-169.

JANCOVICH, J. K., DAVIDSON, E. W., MORADO, J. F., JACOBS, B. L. & COLLINS, J. P., 1997. - Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. *Dis. aquat. Organ.*, **31**: 161-167.

JOSEPH, S. W. & CARNAHAN, A., 1994. The isolation, identification, and systematics of the motile *Aeromonas* species. *Annual Rev. Fish Dis.*, **4**: 315-343.

JUNGWIRTH, T. (ed.), 2004. *Report on the status and conservation of the boreal toad (* Bufo boreas boreas *) in the southern Rocky Mountains 2003*. Colorado Division of Wildlife, 6060 Broadway, Denver, CO 80216.

KIESECKER, J. M. & BLAUSTEIN, A. R., 1995. - Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. *Proc. natl. Acad. Sci. USA*, **92**: 11049-11052.

KIESECKER, J. M. & BLAUSTEIN, A. R., 1997. – Influences of egg laying behavior on pathogenic infection of amphibian eggs. *Conserv. Biol.*, **11** (1): 214-220.

LONGCORE, J. E., PESSIER, A. P. & NICHOLS, D. K., 1999. - *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia*, **91**: 219-227.

MOREHOUSE, E. A., JAMES, T. Y., GANLEY, A. R. D., VILGALYS, R., BERGER, L., MURPHY, J. P. & LONGCORE, J. E., 2003. Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. *Mol. Ecol.*, **12**: 395-403.

MUTHS, E., CORN, P. S., PESSIER, A. P. & GREEN, D. E., 2003. Evidence for disease related amphibian decline in Colorado. *Biol. Conserv.*, 110: 357-365.

OKAFOR, J. I., TESTRAKE, D., MUSHINSKY, H. R., & YANGCO, B. G., 1984. – A *Basidiobolus* sp. and its association with reptiles and amphibians in southern Florida. *J. med. vet. Mycol.*, **22**: 47-51.

O'SHEA, P., SPEARE, R. & THOMAS, A. D., 1990. Salmonellas from the cane toad, *Bufo marinus*. *Aust. vet. J.*, 67 (8): 310.

PALMER, B. D. & SELCER, K. W., 1996. – Vitellogenin as a biomarker for xenobiotic estrogens: a review. *In* D. A. BENGTSON & D. S. HENSHEL (ed.), *Environmental toxicology and risk assessment: biomarkers and risk assessment*, Vol. 5, West Conshohocken, Pennsylvania, ASTM: 3-22.

RITTMANN, S., MUTHS, E. & GREEN, D. E., 2003. - *Pseudacris triseriata* (western chorus frog) and *Rana sylvatica* (wood frog). Chytridiomycosis. *Herp. Rev.*, 34 (1): 53.

Source : MNHN, Paris

REED, K. D., RUTH, G. R., MEYER, J. A. & SHUKLA, S. K., 2000. *Chlamydia pneumoniae* infection in a breeding colony of African clawed frogs (*Xenopus tropicalis*). *Emerging infectious Diseases*, **6** (2): 196-199.

REEDER, A. L., FOLEY, G. L., NICHOLS, D. K., HANSEN, L. G., WIKOFF, B., FAEH, S., EISOLD, J., WHEELER, M. B., WARNER, R., MURPHY, J. E. & BEASLEY, V. R., 1998. Forms and prevalence of intersexuality and effect of environmental contaminants on sexuality in cricket frog (*Acris crepitans*). *Environ. Health Persp.*, **106** (5): 261-266.

SHARMA, V. K., ROHDE, R., GARG, D. N. & KUMAR, A., 1977. Toads as natural reservoir of *Salmonella*. *Zbl. Bakt. Parasit.*, 239: 172-177.

TAYLOR, S. & MILLS, K. W., 1999. Mortality of captive Canadian toads from *Basidiobolus ranarum* mycotic dermatitis. *J. Wildlife Diseases*, **35** (1): 64-69.

TAYLOR, S., WILLIAMS, E. S. & MILLS, K. W., 1999*b*. Experimental exposure of Canadian toads to *Basidiobolus ranarum*. *J. Wildlife Diseases*, **35** (1): 58-63.

TAYLOR, S., WILLIAMS, E. S., THORNE, E. T., MILLS, K. W., WITHERS, D. I. & PIER, A. C., 1999*a*. – Causes of mortality in Wyoming toads. *J. Wildlife. Diseases*, **35** (1): 49-57.

TAYLOR, S. K., GREEN, D. E., WRIGHT, K. M. & WHITAKER, B. R., 2001. – Chapter 13. Bacterial diseases. *In* K. M. WRIGHT & B. R. WHITAKER (ed.), *Amphibian medicine and captive husbandry*, Malabar, Florida, Krieger Publishing Comp.: 159-191.

WAAIJ, D. VAN DER, COHEN, J. B. & NACE, G. W., 1974. – Colonization patterns of aerobic gram-negative bacteria in the cloaca of *Rana pipiens*. *Lab. Anim. Sci.*, **24** (2): 307-317.

WILLIAMS, R. W., 1960. – Observations on the life history of *Rhabdias sphaerocephala* Goodey 1924 from *Bufo marinus* L., in the Bermuda Islands. *J. Helminthol.*, **34**: 93-98.

WRIGHT, K., 1995. Blood collection and hematological techniques in amphibians. *Bull. Assoc. Rept. Amphib. Vet.*, **5** (2): 8-10.

Corresponding editor: C. Kenneth DODD, Jr.

© ISSCA 2005

Source : MNHN, Paris

Alytes, 2005, **22** (3-4): 130-145.

Toxicity to amphibians of environmental extracts from natural waters in National Parks and Fish and Wildlife Refuges

Christine M. Bridges & Edward E. Little

US Geological Survey, Columbia Environmental Research Center,
4200 New Haven Road, Columbia, Missouri 65201, USA

Amphibian population declines are not limited to overtly degraded habitats, but often occur in relatively pristine environments such as national parks or wildlife refuges, thus forcing biologists to examine less obvious causes for declines such as the presence of contaminants. The objective of our study was to extract naturally-occurring compounds from amphibian habitats (using semipermeable membrane devices) in three national parks or wildlife refuges (two sites within Sequoia Kings Canyon National Park, Big Bend National Park, and Kenai National Wildlife Refuge), and assess their toxicity to developing larvae using bioassays. Extracts did not cause mortality, so all effects observed were sublethal, influencing life history characteristics. In all three areas studied, amphibians reared in extracts from at least one of the two sites exhibited either a lengthened larval period or reduced mass at metamorphosis. Extracts from both the air and water at one site in Sequoia Kings Canyon National Park lengthened the larval period, which is in agreement with studies showing elevated levels of aerially transported contaminants at sites such as this within the park. Ultraviolet radiation, which is also suspected of having caused amphibian declines and was included as a factor in our study, did not act alone or alter the toxicity of the extracts.

Introduction

Reports of amphibian population declines in seemingly pristine environments, particularly in the western United States, have become increasingly common (Carey, 1993; Fellers & Drost, 1993, Bradford et al., 1994; Corn, 1994, Jennings & Hayes, 1994, Knapp & Matthews, 2000) Although in some areas of the west the status of amphibian populations remain largely unstudied (e g , along the Rio Grande river, Jung et al., 2002), declines have been documented throughout the Sierra Nevada mountains (Bradford et al., 1993), in western Colorado (Carey, 1993) and in the Cascade mountains (McAllister et al., 1993), among other locations. Although few declines have been definitively linked with causes, others cases have been more clear (Knapp & Matthews, 2000).

Source : MNHN, Paris

Environmental contamination has been proffered as having contributed to some of these declines. For instance, DAVIDSON et al (2001, 2002) found a strong positive relationship between observed patterns of declines in a number of species in the Sierra Nevada mountains and upwind agricultural land usage in the Central Valley of California. SPARLING et al. (2001) correlated a reduction in cholinesterase levels (which may indicate exposure to certain types of toxicants) with areas of population decline in the Sierra Nevadas. It is often difficult, however, to determine what specific contaminants are present at a site or in what concentrations, which often hinders determining the influence of exposure to contaminants on the populations of organisms inhabiting the area. Furthermore, several environmental variables (e.g , pH, Dissolved Oxygen Content, predators) may be acting alone or in combination with the contaminants, contaminants may be present in complex mixtures, and/or the identity and concentrations of specific substances and their metabolites within the composition of such mixtures may be unknown or difficult to determine.

One of our goals was to expose developing amphibian larvae to composite samples of waterborne contaminants in various sites within national parks and fish and wildlife refuges, using in-laboratory bioassays. To accomplish this, we deployed semipermeable membrane devices. These are integrative samplers containing lipid that accumulates lipophilic organic compounds from the environment in a manner that is similar to aquatic organisms (HUCKINS et al., 2002) The uptake rates of fat-soluble compounds by semipermeable membrane devices can be used to define the approximate daily exposure to lipophilic compounds by aquatic organisms (HUCKINS et al., 2002). Thus, the composite waterborne environmental contaminant exposure of organisms to the bioavailable lipophilic compounds present at the site can be accomplished by using extracts from semipermeable membrane devices as toxicant solutions for bioassays (PETTY et al., 2000; HUCKINS et al., 2002) based on the duration of deployment in the environment. Using semipermeable membrane device extracts in bioassays has been shown to be an effective way to assess the toxicity and teratogenicity of naturally occurring environmental compounds to larval anurans (BRIDGES & LITTLE, 2003; BRIDGES et al., 2004)

Ultraviolet (UV) radiation has also been suspected of causing declines among amphibian populations. Exposure to UVB radiation at larval and egg stages has been suspected of negatively impacting some amphibian species directly by reducing hatching success and increasing rates of embryonic malformation (BLAUSTEIN et al , 1998, LIZANA & PEDRAZA, 1998, BROOMHALL et al , 2000, STARNES et al., 2000) as well as generating larval malformations (ANKLEY et al., 2000). And although being a lesser factor than contaminants, DAVIDSON et al. (2002) suspected UV as a likely contributor to amphibian declines in the Sierra Nevada mountains. However, because no environmental factor acts in isolation, it has become clear that single-factor explanations simply may not be sufficient to explain this widespread phenomenon of population declines. For example, UV radiation is also known to increase the toxicity and/or teratogenicity to amphibians of various compounds in aquatic habitats (ZAGA et al , 1998; LITTLE et al., 2000; BRIDGES et al., 2004)

One of our objectives was to investigate whether exposure to lipophilic compounds extracted from natural environments located in conventionally pristine areas (i e., Sequoia Kings Canyon National Park, Big Bend National Park, Kenai National Wildlife Refuge) can negatively affect developing amphibian larvae. Furthermore, because UV radiation can also

Source : MNHN, Paris

impact amphibians (in and of itself as well as when in combination with environmental contaminants), the effects of UV radiation were also explored. In this study we examined the effects of these two factors, and their interaction, on amphibian larval survival, length of the larval period, and size at metamorphosis for various anuran species.

Materials and methods

Semipermeable membrane device exposure and extraction

To determine whether contaminants present at our study sites can cause mortality or malformations in larval amphibians, we deployed semipermeable membrane devices at two sites within each refuge or national park. Five standard semipermeable membrane devices (described in Huckins et al., 2002) were placed in each of three replicate stainless-steel canisters attached to a steel cable anchored to the shore. The conditions of each deployment are given below.

At the time of deployment a metal can containing semipermeable membrane devices (hereafter, field blank) was opened and exposed to the air at each site. These provided a control for any airborne contaminants present while the semipermeable membrane devices were exposed to the air (i.e., before being placed into the water) Once the semipermeable membrane devices were in the water, the cans containing the field blanks were sealed and stored at 0-4°C for the time between semipermeable membrane device deployment and retrieval to halt contaminant uptake (from the site air that had filled the can). During retrieval, field blanks were once again exposed to the air at the site for as long as it took to remove the semipermeable membrane devices from the water and seal them in cans. After retrieval, both semipermeable membrane devices and field blanks were shipped on ice and remained frozen until they were processed at the Columbia Environmental Research Center (Columbia, Missouri) using techniques outlined in Petty et al. (2000).

All semipermeable membrane device extracts from each site were pooled into a single composite sample. Field exposure and control extracts (i.e., field blanks) were dissolved into sterile dimethylsulfoxide (DMSO) by solvent exchange. Semipermeable membrane device extracts were added to 90 ml of sterile DMSO so that each ml contained the approximate equivalent of a 1-d exposure (i e., representing the amount of bioavailable residues extracted from site water by a standard semipermeable membrane devices in one days' time) when added to 1 l of water

Assuming lentic conditions, an ambient temperature between 10 and 18° C, and minimal biofouling, 1-gram equivalent of a standard 1-ml triolein semipermeable membrane devices will clear or extract hydrophobic organic contaminants (e g , PAH, PCB, organochlorines, pyrethroids) from about 0.01 to 2.0 l/g of water daily (Huckins et al , 2002) Although much greater variability exists in the uptake rate constants of aquatic organisms for the same chemicals, the values for invertebrates and fishes generally range from 0.03 to 8 0 l,d $^{-1}$g $^{-1}$ (Mackay et al., 1991, 1992, 1997). Thus, it is reasonable to expect that aliquots of semipermeable membrane device extracts, which represent the daily volume of water extracted by a

Source : MNHN, Paris

whole standard semipermeable membrane devices, are representative of the amounts of chemicals to which aquatic organisms (e g., tadpoles) are exposed daily.

COLLECTION SITES AND CONDITIONS

Because our semipermeable membrane devices were deployed during various times of the year, the extracts may not be composed of the same compounds that might be available during the spring, when many amphibian species breed. However, semipermeable membrane devices are designed to sample very persistent contaminants (PETTY et al , 2000), and whereas the concentrations of the waterborne lipophilic contaminants may change with increased runoff, etc , the composition of these contaminants would likely change very little. Consequently, sampling the water in the late summer/early fall likely represents a conservative effects assessment. Regardless of when our semipermeable membrane devices were deployed, the time of deployment coincides with at least partial development of most species in these habitats.

We attempted to choose sites within each park or national refuge that were disparate in their habitats (e g , one high and one low elevation), initially wishing to select one site that would be more contaminated than the other. Species used within two of the three tests are species found within our sampling area. In one case (i.e , Alaskan sites), however, this was not possible and we used a surrogate species.

Sequoia and Kings Canyon National Park

The first site within Sequoia and Kings Canyon National Park was at Yucca Creek, just east of the western border of the park It is a mostly shaded, slowly flowing, clear stream with a rocky bottom. Semipermeable membrane devices were deployed at approximately 1 m underwater Water temperature was 16°C when the devices were deployed on 12 September 2000 and when they were retrieved on 11 October 2000 Upon retrieval there was no evidence that the devices had been disturbed

The second site within Sequoia and Kings Canyon National Park was in Aster Lake, a clear, lentic alpine lake at about 2800 m elevation approximately 8 km west of the Wolverton trail head within the park The site is not shaded and the semipermeable membrane devices rested 1 5 m underwater The water temperature was 10°C when the devices were deployed on 13 September 2000 and 12°C upon retrieval on 12 October 2000. Upon retrieval there was no evidence that the devices had been disturbed.

Big Bend National Park

The first site within Big Bend National Park was in the Rio Grande River approximately three miles from Castalon The water, which was unshaded, muddy and had a moderate flow, was 23°C upon deployment of the devices on 4 April 2001 We are uncertain as to the exact depth of deployment but anticipate it was at least 2 m Upon deployment, the amount of UVA transmitted through the water column at a depth of 10 cm was 9,700 uW/cm^2 and the UVB was 10 3 uW/cm^2 when measured with a Macam Photometrics broadband UV meter Upon

Source : MNHN, Paris

retrieval on 10 May 2001 the water temperature was 24°C and the devices had a substantial amount of mud and grime (i e , biofouling) coating them, but there was no evidence of them having been disturbed.

The second site within Big Bend National Park was at Lower Cattail Falls, at the end of a trailhead, the entrance of which is across the street from the Sam Nail Ranch. This site is a shaded clear flowing stream and the device was anchored in 1 m of water. The water temperature was 19 8°C when the devices were deployed on 5 April 2001 and was 21°C when the devices were retrieved on 10 May 2001. Upon deployment, the amount of UVA transmitted through the water column at a depth of 10 cm was 1,182 uW/cm^2 and the UVB was 40.4 uW/cm^2 when measured with a Macam Photometrics broadband UV meter There was no evidence of biofouling or disturbance.

Kenai National Wildlife Refuge

Both sites within the Kenai National Wildlife Refuge are located within the Swanson River unit Both are boggy, unshaded wetlands adjacent to oil fields. All devices were deployed on 11 July 2001 and retrieved on 8 August 2001. At Oil Field 1 (Parsons, 2001) the water temperature was 16°C when the devices were deployed and 21°C when they were retrieved. Upon deployment, the amount of UVA transmitted through the water column at a depth of 10 cm was 2,190 uW/cm^2 and the UVB was 22.6 uW/cm^2 when measured with a Macam Photometrics broadband UV meter. At our second site, Oil Field 3 (Parsons, 2001), the water temperature was 18°C at deployment and 19°C at retrieval. Upon deployment, the amount of UVA transmitted through the water column at a depth of 10 cm was 2,940 uW/cm^2 and the UVB was 46.2 uW/cm^2 When each set of devices was collected, no biofouling had occurred and there was no evidence of tampering

Exposure to semipermeable membrane device extracts

Exposures were carried out over two years due to space and time limitations. In June 2001, Pacific treefrog (*Pseudacris regilla*) tadpoles from three egg masses were received from Sunriver, Oregon to be used in tests with extracts from Sequoia and Kings Canyon National Park. The exposure began on 8 June 2001 and was completed 40 d later when the last tadpole reached metamorphosis. In April 2002, three spring peeper (*Pseudacris crucifer*) egg masses were collected from a farm pond in Boone County, Missouri to use in the tests of extracts from Kenai National Wildlife Refuge. This exposure began on 20 April 2002 and ended 51 d later when the last tadpole reached metamorphosis. We selected the spring peeper as a surrogate test species for the native wood frog because of its availability from an uncontaminated habitat, its comparatively short developmental time to metamorphosis, and its high sensitivity to environmental contaminants. Birge et al. (2000) ranked the spring peeper as highly sensitive based upon acute toxicity tests of 34 inorganic elements and 27 organic chemicals. In these tests the spring peepers consistently exhibited lower tolerance for such exposures than any of the ranid species tested. Thus, any results obtained using this species should yield a conservative estimate of effects that wood frogs would experience In June 2002, canyon treefrog (*Hyla arenicolor*) tadpoles were collected from Big Bend National Park to be

Source : MNHN, Paris

used in exposures to extracts from that park The exposure began on 21 June 2002 and was completed 45 d later when the final tadpole reached metamorphosis.

The experiment was designed as a 2 × 5 complete factorial: tadpoles were exposed to one of two UV light treatments and to one of five semipermeable membrane device treatments (see below), replicated three times.

Test solutions were created by filling 3-l beakers with 2 l of well water, and adding 1.0 ml of the appropriate semipermeable membrane device extract treatment (hereafter, semipermeable membrane devices treatment) or field blank semipermeable membrane devices. We also used a well water (pH 7.8; hardness 286 mg/l $CaCO_3$; alkalinity 258 mg/l $CaCO_3$) control.

Our high UV treatment (16.2 $\mu W/cm^2$ UVB) was achieved by wrapping the sides of each chamber in polycarbonate plastic (0.030 inch thickness, Cope Plastics, Inc., St Louis, Missouri) and covering the top of the chamber with two pieces of cellulose acetate (0 015 inch thickness, Cope Plastics, Inc., St. Louis, Missouri) and one piece of and shade cloth (50 % shading, Lowe's Home Improvement Center). The low UV treatment (<1 $\mu W/cm^2$ UVB) was created by wrapping the sides of the chambers with Mylar D (0.005 inch thickness, Cope Plastics, Inc , St Louis, Missouri) and covering the tops of the chambers with two pieces of polycarbonate plastic and one piece of shade cloth.

Prior to testing, the irradiance level of each treatment was measured with a spectroradiometer at a 10-cm depth in a solar-simulating chamber, as described in LITTLE & FABACHER (1996). The high irradiance value we used throughout the experiment in the solar simulator fell below the mean value we measured in the field at a 10 cm depth. This corresponds to the depth at which many egg masses are found and where they can be found thermoregulating and feeding. The simulator was programmed on a 16L:8D photoperiod. UVB lights were activated five hours into the light cycle for five hours to simulate midday solar intensity. This lighting regime provides for the induction of cellular photorepair functions and prevents the over-estimation of UV-induced injuries.

Groups of three tadpoles were placed in the 3-l jars randomly arranged under a solar simulator in a 20°C flow-through water bath Tadpoles were exposed to one of five semipermeable membrane device treatments: (1) a well water control, (2) semipermeable membrane device extract from the first site in the refuge/national park; (3) field blank extract from the first site; (4) semipermeable membrane device extract from the second site within the refuge/national park; and (5) field blank extract from the second site Each time water was changed (i e., every third day), 1 ml of the appropriate extract (the equivalent to a 2-d dose) was added to the jars. Tadpoles were fed ground fish flakes (Tetra-Min® brand) ad libitum at each test water change until metamorphosis, which was defined as the emergence of at least one forelimb (stage 42, GOSNER, 1960) At metamorphosis individuals were removed from the experimental chamber and housed in the laboratory until tail resorption (about 4 d, or stage 46, GOSNER,1960), when they were weighed to the nearest 0 1 mg.

STATISTICAL ANALYSES

Values for the three tadpoles in each replicate chamber were pooled to attain a single data point For each location we used analysis of variance (ANOVA) to determine whether mass at

Source : MNHN, Paris

metamorphosis and the length of the larval period were dependent on semipermeable membrane device extract treatment, UV treatment, or the interaction of these factors. Both endpoints were log-transformed to increase normality In analyses of mass at metamorphosis, length of the larval period was used as a covariate and vice versa because these two variables can be correlated. In the instances where the type I sums of squares of these covariates were not significant, they were removed from the model. When there were significant differences among semipermeable membrane device extract treatments, Bonferroni multiple comparison tests were conducted to discern differences among specific treatments at $\alpha = 0.05$ Differences in survival were minimal (i e., nearly all animals survived through metamorphosis), so no analyses were conducted on this endpoint.

Results

In Sequoia and Kings Canyon National Park, the effect of extract treatment on the size at metamorphosis of tadpoles was marginally significant (tab. 1). Individuals reared in the water controls were significantly larger at metamorphosis than tadpoles in extracts from Yucca Creek (fig 1). However, Yucca Creek tadpoles were not significantly smaller than tadpoles reared in extracts from Aster Lake, or in either of the field blank treatments. The effect of extract treatment on the length of the larval period was not significant However, when the effects of UV (which were not significant) were removed from the model, the main effect of semipermeable membrane device extract treatment became marginally significant ($F_{4,24} = 2{,}58$, $P = 0.0634$). Tadpoles reared in SPMD extracts from Aster Lake and in extracts from the Aster Lake field blank took longer to reach metamorphosis than those from any other treatment (fig. 2). Neither UV treatment alone nor the interaction between the UV treatment and the semipermeable membrane device extract treatment significantly affected the length of the larval period (tab. 1) or the mass at metamorphosis.

In Big Bend National Park, semipermeable membrane device extract treatment significantly affected the number of days it took for tadpoles to reach metamorphosis (tab. 1) Individuals reared in water containing extracts from the Rio Grande River took significantly longer to transform than individuals reared in field blanks from either site, or controls (fig 3) The length of the larval period in tadpoles reared in water from Lower Cattail Falls was not significantly different than those from the Rio Grande River, and did not differ from controls or field blanks from either site (fig. 3).

At Kenai National Wildlife Refuge, semipermeable membrane device extract treatment significantly affected the length of the larval period (tab 1) Individuals reared in semipermeable membrane device extracts from Oil Field 3 took significantly longer to reach metamorphosis than individuals reared with Oil Field 3 field blanks, Oil Field 1 semipermeable membrane device extracts, or the control (fig 4). The length of the larval period was not significantly affected by UV treatment or the interaction between semipermeable membrane device treatment and UV Semipermeable membrane device extracts from Kenai National Wildlife Refuge also significantly affected the size at metamorphosis for tadpoles. Individuals reared in extracts from Oil Field 3 were significantly smaller than those reared in any other

Source : MNHN, Paris

Table 1. – Analyses of variance on the effects of SPMD extract (extract), UV radiation (UV) and their interaction on the length of the larval period (days) and the mass at time to metamorphosis (mass) for amphibian larvae. Type III mean squares (*MS*) are reported BBNP, Big Bend National Park; KNWR, Kenai National Wildlife Refuge; SEKI, Sequoia Kings Canyon National Park.

Response variable	Source	*df*	*MS*	*F*	*P*
Days (SEKI)	Extract	4	0 0053	1.80	0.1705
	UV	1	0 0013	0 47	0 5032
	Extract × UV	4	0 0010	0.35	0 8403
	Error	19	0 0029		
Mass (SEKI)	Extract	4	0 0509	2.67	0 0637
	UV	1	0 0174	0.92	0.3505
	Extract × UV	4	0 0086	0.45	0.7690
	Error	19	0.0190		
Days (BBNP)	Mass	1	0.0058	2.02	0.1718
	Extract	4	0 0122	4.25	0 0126
	UV	1	0 0022	0.76	0 3930
	Extract × UV	4	0 0014	0.48	0 7481
	Error	19	0.0029		
Mass (BBNP)	Days	1	0 0295	1.72	0 2053
	Extract	4	0.0217	1.57	0 2223
	UV	1	0.0035	0.21	0 6549
	Extract × UV	4	0.0019	0.12	0 9751
	Error	19	0.0172		
Days (KNWR)	Mass	1	32.39	6 15	0.0227
	Extract	4	15.61	2.96	0.0464
	UV	1	1 19	0.23	0 6394
	Extract × UV	4	7.99	1.52	0 2371
	Error	19	5.26		
Mass (KNWR)	Days	1	0 08	6.31	0 0212
	Extract	4	0.05	3.72	0.0212
	UV	1	0.00	0.07	0.7887
	Extract × UV	4	0 03	2.25	0.1020
	Error	19	0 01		

Source : MNHN, Paris

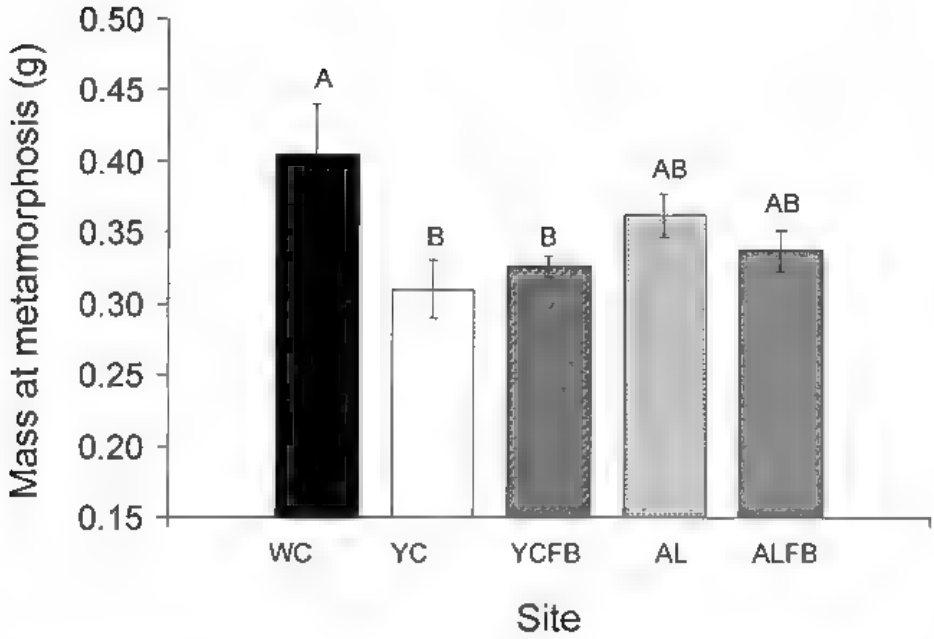


Fig 1 - Mass at metamorphosis for *Pseudacris regilla* tadpoles reared in each treatment for Sequoia Kings Canyon National Park AL, Aster Lake, ALFB, Aster Lake Field Blank, WC, water control; YC, Yucca Creek; YCFB, Yucca Creek Field Blank. Vertical lines represent ± 1 s_x.

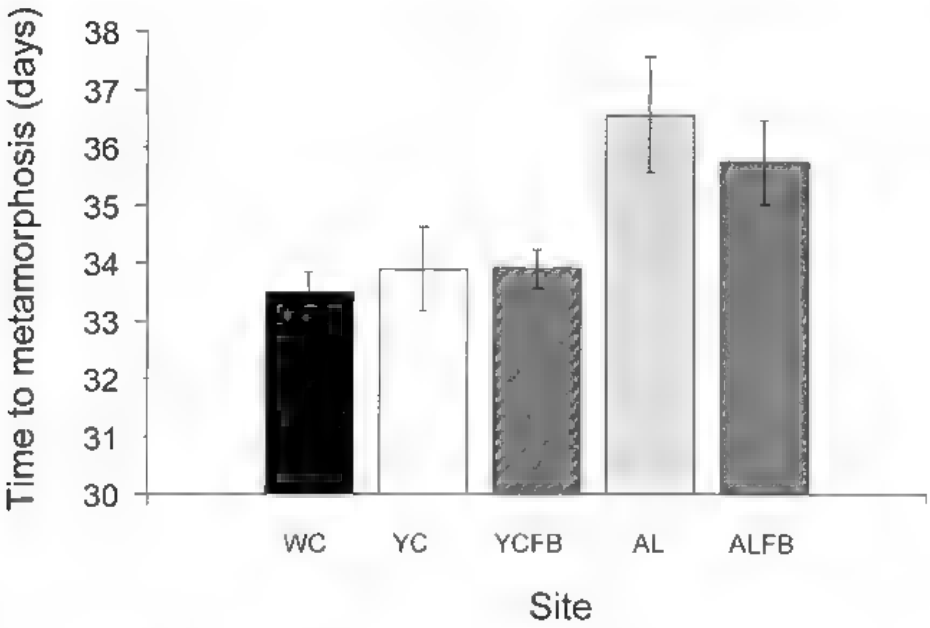


Fig 2 Time to metamorphosis in days for *Pseudacris regilla* tadpoles reared in each treatment for Sequoia Kings Canyon National Park AL, Aster Lake, ALFB, Aster Lake Field Blank, WC, water control, YC, Yucca Creek, YCFB, Yucca Creek Field Blank Bars with the same letters do not differ significantly from one another. Vertical lines represent ± 1 s_x

Source : MNHN, Paris

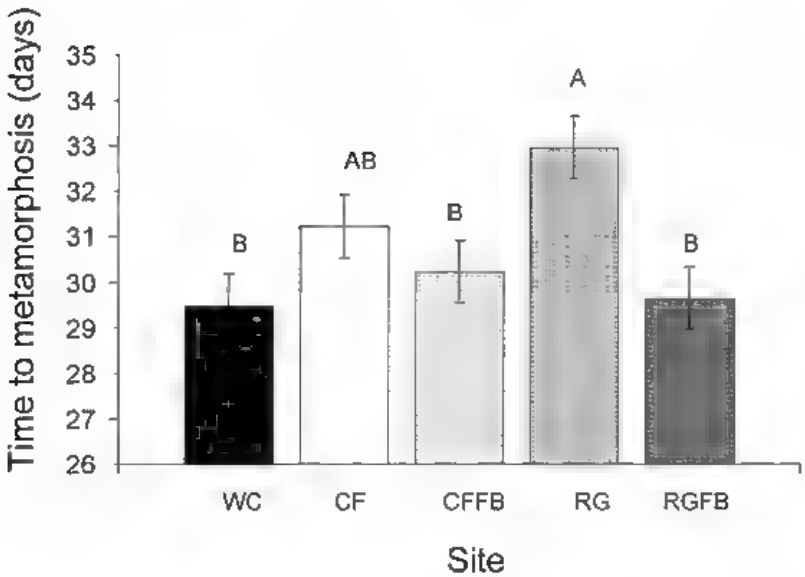


Fig 3 Time to metamorphosis in days for *Hyla arenicolor* tadpoles reared in each treatment for Big Bend National Park. CF, Cattail Falls, CFFB, Cattail Falls Field Blank, RG, Rio Grande; RGFB, Rio Grande Field Blank; WC, water control Bars with the same letters do not differ significantly from one another. Vertical lines represent ± 1 s_x.

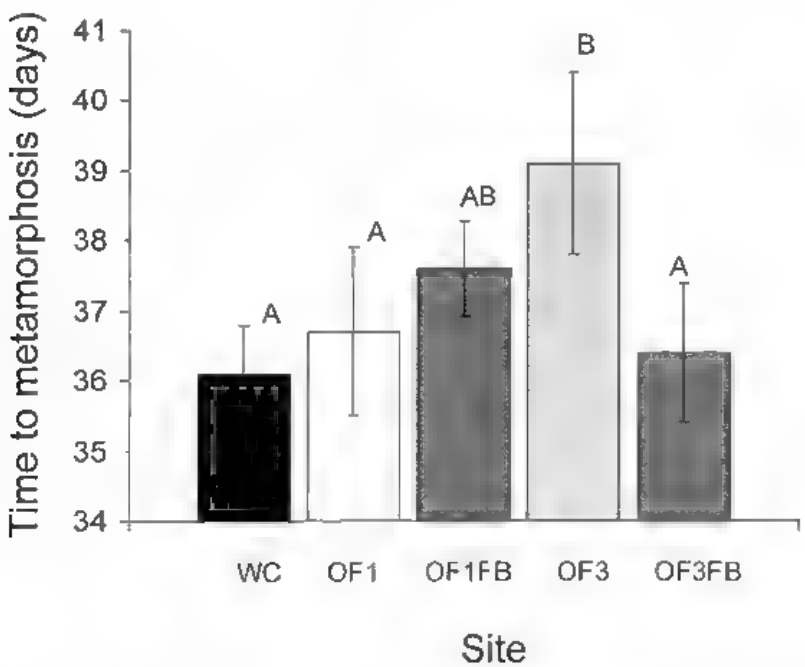


Fig 4 Time to metamorphosis in days for *Hyla arenicolor* tadpoles reared in each treatment for Kenai National Wildlife Refuge. OF1, Oil Field 1, OF1FB, Oil Field 1 Field Blank, OF3, Oil Field 3, OF3FB, Oil Field 3 Field Blank, WC, water control Bars with the same letters do not differ significantly from one another. Vertical lines represent ± 1 s_x

Source : MNHN, Paris

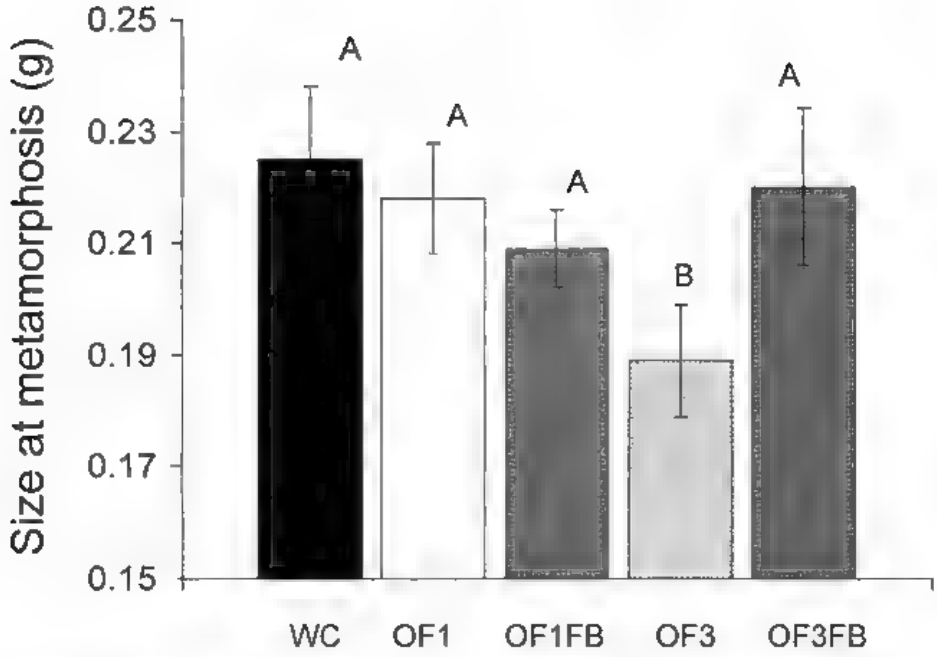


Fig. 5 Mass at metamorphosis in days for *Pseudacris crucifer* tadpoles reared in each treatment for Kenai National Wildlife Refuge. OF001, Oil Field 1, OF001FB, Oil Field 1 Field Blank, OF003, Oil Field 3; OF003FB, Oil Field 3 Field Blank, WC, water control. Bars with the same letters do not differ significantly from one another. Vertical lines represent $\pm$ 1 $s_{\bar{x}}$.

treatment (fig. 4). There was no effect of UV radiation or the interaction between UV radiation and semipermeable membrane device extract treatment on the size at metamorphosis.

Discussion

Utilizing semipermeable membrane device extracts in amphibian bioassays has been shown to be an effective tool in determining whether lipophilic compounds found in aquatic amphibian habitats are toxic (Bridges et al., 2004) without actually having to determine which compounds are present. Importantly, using semipermeable membrane devices rules out detrimental effects attributable to parasites or pathogens because the pore size of the membrane is too small (i.e., 10 Å) to allow passage of bacterial, viral or fungal cells. Although we did not attempt to determine which compounds were present in the extracts used in our experiments, once the presence of contaminants in the environment are confirmed in a bioassay it would be possible to resample the sites and generate a contaminant profile.

Despite the fact that Sequoia and Kings Canyon National Park is a protected, natural area, the presence of contaminants has been revealed throughout the park. Surveys of the

Source : MNHN, Paris

Sierra Nevada mountains around Sequoia and Kings Canyon National Park indicate the presence of chlorpyrifos, malathion, diazanon and DDT in water (ZABIK et al, 1993; MCCONNELL et al., 1998) and in frog tissue (CORY et al., 1970) even at high altitudes within the park. SPARLING et al. (2001) revealed reduced cholinesterase levels of native amphibians in Sequoia Kings Canyon National Park, suggesting the presence of cholinesterase-inhibiting pesticides. DAVIDSON et al (2001, 2002) correlated the amount of downwind pesticide use in California's Central Valley with declines in amphibian populations within the park LENOIR et al (1999) have shown the presence of diazanon and chlorpyrifos in atmospheric transport at elevations where native amphibians are showing the greatest declines.

Our data using Pacific treefrogs (*P. regilla*, which are native to the Sequoia and Kings Canyon National Park region) suggests the presence of a compound in the water at Yucca Creek that generates a smaller size at metamorphosis, which can have a number of fitness consequences (ALTWEGG & REYER, 2003) Individuals that are large at metamorphosis can realize higher overwintering success, greater survival to first reproduction, and earlier reproduction (SMITH, 1987; SEMLITSCH et al., 1988). Further, larger females can carry more eggs, and larger males often gain access to a greater number of females during breeding, leading to increased reproductive success (BERVEN, 1982). The fact that there were no significant differences when comparing Yucca Creek with the other treatments may suggest the presence of a compound in all of our semipermeable membrane device extracts and field blanks that depresses growth (although not detectable significantly in the other treatments).

There was a trend for frogs reared in extracts from Aster Lake and the Aster Lake field blank to take longer to reach metamorphosis. This effect was marginally significant only after the removal of UV effects, perhaps because our statistical power was low. That reductions were observed both in the field blank and the waters extracts from this high elevation site suggests the presence of a compound that is airborne and/or waterborne. This is in agreement with data that indicate the presence of contaminant compounds at high elevations in the Sierra Nevadas suspected of causing declines (LENOIR et al, 1999). Frogs delaying metamorphosis can suffer some of the same fitness detriments as animals undergoing metamorphosis at a small size.

In 2002, Big Bend National Park was designated an endangered park primarily due to its increasingly high levels of human impacts over the past 15 years. Poor air quality attributable to drift from coal-fired power plants is common Increasing aquatic pollution and water diversion upstream has caused the water quality to decline in the Rio Grande River, which is the park's most prominent source of water In fact, low water levels lead to concentration of aquatic pollutant from upstream municipal and agricultural sources in the US and Mexico, further worsening the quality of aquatic habitat in this river.

Metamorphosis was delayed among canyon treefrog (*H arenicolor*) tadpoles, which are native to Big Bend National Park, developing in water containing extracts from the Rio Grande River Levels of lipophilic contaminants (including DDT) have been detected at sampling sites upstream and downstream of the park (SCHMITT et al., 2004) and in biota feeding in this stretch of the river (MORA et al, 2002). This suggests that amphibian larvae developing in the Rio Grande, or in pools formed by its floodwaters, are at a potential disadvantage which may result in decreased fitness. Tadpoles reared in extracts from Cattail Falls did not take significantly longer to develop than tadpoles in the Rio Grande, but also did

Source : MNHN, Paris

not differ from the controls. No effects were seen due to extracts from the field blanks, indicating that the contaminants sampled at each site were waterborne.

The Kenai National Wildlife Refuge covers nearly 2 million square acres on Alaska's Kenai Peninsula, south of Anchorage. In recent years, activities have taken place on the Kenai National Wildlife Refuge that have increased chemical contamination on this site including oil and gas development, pesticide application, military and recreational uses, mining, and use of fire retardants (Parsons, 1991). Many of these sites were impacted with little or no post-contamination remediation efforts undertaken, primarily because of Alaska's remoteness. We selected two ponds on the Kenai National Wildlife Refuge adjacent to oil drilling operations and that also contain populations of wood frogs (*Rana sylvatica*).

At Kenai National Wildlife Refuge, frogs exposed to semipermeable membrane device extract from the pond at Oil Field 3 were smaller at metamorphosis and took longer to reach metamorphosis than frogs exposed to any other treatment. Why there were effects at Oil Field 3 and not Oil Field 1, which are both adjacent to oil drilling operations, is unclear. The fact that the growth and development of the frogs reared in extract from the Oil Field 3 field blank did not differ from the water control indicates that the contaminant in question is aquatic and not airborne. Whereas a number of petroleum hydrocarbon spills have been documented in the Swanson River Oil Field (Parsons, 2001), where our two sites were situated, it is unclear whether any of these historic spills occurred near our study sites. Without chemical analysis of the semipermeable membrane device extracts, it is not known what chemicals may have elicited this biological response, nor whether this phenomenon is related to oil field operations. Hydrocarbons, among other chemicals, have been shown to be made more toxic in the presence of UV radiation (Zaga et al., 1998, Little et al., 2000). Because there was not a significant interaction between the semipermeable membrane device extracts and UV radiation, it is likely that hydrocarbons are not a significant source of contamination at these two sites.

We did not observe any effects of UV radiation alone within our study, suggesting that the intensities of UV which were measured in the field are not high enough to singly harm amphibians. There was also no interaction between UV and semipermeable membrane device extracts from any site. This indicates that the compounds present in these waters are not broken down or photoactivated to be made less or more toxic, respectively.

Conclusions

Habitat decimation has been cited as being the major contributor of amphibian declines, but when declines are occurring in protected areas we are forced to consider other, more insidious, factors such as contamination. Often, habitats that appear pristine can be unfavorable for amphibians and other aquatic organisms. In our study, alterations in amphibian life history characteristics were evident within all three of the parks and wildlife refuges examined. Even if contaminants do not cause outright mortality, alterations in life history characteristics have the potential to alter population structure over time. For example, if postponing metamorphosis delays age at first reproduction, population growth rates

Source : MNHN, Paris

potentially decrease (STEARNS, 1992). If in a contaminated environment there were a shift toward later reproduction in an amphibian population due to delayed maturity, the demographic structure of the population may be altered, resulting in a gradual decline in size over time. Utilizing semipermeable membrane devices offers a way to sample aquatic habitats for contaminants in a non-destructive manner, and can be used to help assess the health of aquatic amphibian habitats.

RÉSUMÉ

Les declins des populations d'amphibiens ne sont pas limités aux habitats ouvertement dégradés, mais s'observent souvent dans des environnements relativement intacts tels que des parcs nationaux ou des réserves naturelles, forçant ainsi les biologistes à examiner des causes de déclin moins évidentes a priori, comme la présence de contaminants. L'objectif de notre étude était d'extraire, au moyen de membranes semi-perméables, des composés présents dans des habitats naturels d'amphibiens dans trois parcs nationaux ou réserves naturelles (Sequoia Kings Canyon National Park, Big Bend National Park et Kenai National Wildlife Refuge; deux sites étudiés pour chacun), et d'évaluer leur toxicité par des tests biologiques sur des larves. Les extraits ne causèrent pas de mortalité, et tous les effets observés furent sublétaux, modifiant des caractéristiques du déverloppement. Dans les trois régions étudiées, les amphibiens élevés avec des extraits provenant d'au moins un des deux sites ont manifesté soit un allongement de la période larvaire soit une réduction de la masse à la métamorphose. Les extraits provenant de l'air et de l'eau d'un site du Sequoia Kings Canyon National Park allongèrent la période larvaire, ce qui est en accord avec les résultats d'études montrant des niveaux élevés de contaminants transportés par l'air dans de tels sites au sein du parc. Les radiations ultraviolettes, qui ont également eté soupçonnées d'avoir causé des déclins d'amphibiens, ne se sont pas avérées agir seules ou altérer la toxicité des extraits.

ACKNOWLEDGMENTS

Our thanks go to H. Werner, R. Skiles, J. Goldstein, S. Olson, D. Hughes, R. Caltee, J. Connor and J. Bridges, for assisting in deploying and/or retrieving devices. J. Bowerman, G. Dayton and T. Timock helped in collecting and/or shipping tadpoles to be used in studies. R. Clark, W. Cranor, J. Huckins and J. Petty assisted us with assembling and processing the semipermeable membrane devices. S. Saura Mas, J. Little, J. Wells and M. Boone helped with the set-up and maintenance of the tests. This manuscript was improved with the thoughtful comments of S. James.

LITERATURE CITED

ALTWEGG, R. & REYER, H-U., 2003. Patterns of natural selection on size at metamorphosis in water frogs. *Evolution*, **57** (4): 872-882.

Source : MNHN, Paris

ANKLEY, G. T., TIETGE, J. E., HOLCOMBE, G. W., DEFOE, D. L., DIAMOND, S. A., JENSEN, K. M. & DEGITZ, S. J., 2000. – Effects of laboratory ultraviolet radiation and natural sunlight on survival and development of *Rana pipiens*. *Can. J. Zool.*, **78**: 1092-1100.

BERVEN, K. A., 1982. – The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. I. An experimental analysis of life history traits. *Evolution*, **36**: 962-983.

BIRGE, W. J., WESTERMAN, A. G. & SPOMBERG, J. A., 2000. – Comparative toxicology and risk assessment of amphibians. *In*: D. W. SPARLING, G. LINDER & C. A. BISHOP (ed.), *Ecotoxicology of Amphibians and Reptiles*, Pensacola, Florida, SETAC Press: 727-792

BLAUSTEIN, A. R., KIESECKER, J. M., CHIVERS, D. P., HOKIT, D. G., MARCO, A., BELDEN L. K. & HATCH, A., 1998. – Effects of ultraviolet radiation on amphibians: field experiments. *Am. Zool.*, **38**: 799-812

BRADFORD, D. F., GRABER, D. M. & TABATABAI, F., 1994. – Population declines of the native frog, *Rana muscosa*, in Sequoia and Kings Canyon National Parks, California. *Southwest. Nat.*, **39**: 323-327

BRADFORD, D. F., TABATABAI, F. & GRABER, D. M., 1993. – Isolation of remaining populations of the native frog, *Rana muscosa*, by introduced fishes in Sequoia and Kings Canyon National Parks, California. *Conserv. Biol.*, **7** (4): 882-888.

BRIDGES, C. M. & LITTLE, E. E., 2003. – Using semipermeable membrane devices (SMDPs) to assess the toxicity and teratogenicity of aquatic amphibian habitats. *In*: G. LINDER, S. KREST, D. SPARLING & E. LITTLE (ed.), *Multiple stressor effects in relation to declining amphibian populations. ASTM STP 1443*, West Conshohocken, Pennsylvania, ASTM International.

BRIDGES, C. M., LITTLE, E. E., GARDINER, D. M., PETTY, J. D. & HUCKINS, J. N., 2004. – Assessing the toxicity and teratogenicity of pond water in Minnesota to native amphibian larvae. *Environ. Sci. Poll. Res.*, **11**: 233-239.

BROOMHALL, S. D., OSBORNE, W. S. & CUNNINGHAM, R. B., 2000. – Comparative effects of ambient ultraviolet-B radiation on two sympatric species of Australian frogs. *Conserv. Biol.*, **14**: 420-427

CAREY, C., 1993. – Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. *Conserv. Biol.*, **7**: 355-362.

CORN, P. S., 1994. – What we know and don't know about amphibian declines in the west. *In*: W. W. COVINGTON & L. F. DEBANO (ed.), *Sustainable ecological systems: implementing an ecological approach to land management*, USDA Forest Service Technical Report RM-247: 59-67

CORY, L., FIELD, P. & SERAT, W., 1970. – Distribution patterns of DDT residues in the Sierra Nevada Mountains. *Pest. Mon. J.*, **3**: 204-211.

DAVIDSON, C., SHAFFER, H. B., & JENNINGS, M. R., 2001. – Declines of the California red-legged frog: climate, habitat, and pesticide hypotheses. *Ecol. Appl.*, **11**: 464-479

----- 2002. – Spatial tests of the pesticide drift, habitat destruction, UV-SS, and climate-change hypotheses for California amphibian declines. *Conserv. Biol.*, **16**: 1588-1601

FELLERS, G. M. & DROST, C. A., 1993. – Disappearance of the Cascades frogs, *Rana cascadae*, at the southern end of its range. *Biol. Conserv.*, **65**: 177-181

GOSNER, K. L., 1960. – A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**: 183-190.

HUCKINS, J. N., PETTY, J. D., PREST, H. F., CLARK, R. C., ALVAREZ, D. A., ORAZIO, C. E., LEBO, J. A., CRANOR, W. L. & JOHNSON, B. T., 2002. – *A guide for the use of semipermeable membrane devices (SMDPs) as samplers of waterborne hydrophobic organic contaminants*. Report prepared for the American Petroleum Institute (API), API Nr. 4690: 1-186

JENNINGS, M. R. & HAYES, M. P., 1994. – *Amphibian and reptile species of special concern in California*. Report prepared for California Department of Fish and Game, Inland Fisheries Division, Rancho Cordova, California

JUNG, R. E., BONINE, K. E., ROSENSHIELD, M. L., DE LA RIZA, A., RAIMONDO, S. & DROGE, S., 2002. – Evaluation of canoe surveys for anurans along the Rio Grande in Big Bend National Park, Texas. *J. Herp.*, **36** (3): 390-397

KNAPP, R. A. & MATTHEWS, K. R., 2000. – Non-native fish introductions and the decline of mountain yellow-legged frogs from within protected areas. *Conserv. Biol.*, **14**: 428-438

LE NOIR, J. S., MCCONNELL, L. L., FELLERS, M. G., CAHILL, T. M. & STIBER, J. N., 1999. – Summertime transport of current-use pesticides from California's Central Valley to the Sierra Nevada mountain range, USA. *Environ. toxicol. Chem.*, **18**: 2715-2722.

Source : MNHN, Paris

LITTLE, E. E., CALFEE, R., CLEVELAND, L., SKINKER, R., ZAGA, PARKHURST, A. & BARRON, M. G., 2000. Photo-enhanced toxicity in amphibians: synergistic interactions of solar ultraviolet radiation and aquatic contaminants. *J. Iowa Acad. Sci.*, **107**: 767-771.

LITTLE, E. E. & FABACHER, D. L., 1996. Exposure of freshwater fish to simulated solar UVB radiation. *In* G. OSTRANDER (ed.), *Techniques in aquatic toxicology*, New York, New York, Lewis Publishers: 141-158.

LIZANA, M. & PEDRAZA, E. M., 1998. - The effects of UV-B radiation on toad mortality in mountainous areas of central Spain. *Conserv. Biol.*, **12**: 703-707.

MACKAY, D., SHIU, W.-Y. & MA, K.-C., 1991. *Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals.* Volume **1**. Chelsea, Michigan, Lewis Publishers: 1-704.

----- 1992. *Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals.* Volume **2**. Chelsea, Michigan, Lewis Publishers: 1-608.

----- 1997. *Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals.* Volume **5**. Chelsea, Michigan, Lewis Publishers: 1-832.

MCALLISTER, K. R., LEONARD, W. P. & STORM, R. M., 1993. – Spotted frog (*Rana pretiosa*) surveys in the Puget trough of Washington, 1989-1991. *Northwest Nat.*, **74**: 10-15.

MCCONNELL, L. L., LENOIR, J. S., DATTA, S. & SEIBER, J. N., 1998. – Wet deposition of current use pesticides in the Sierra Nevada mountain range. *Environ. toxicol. Chem.*, **17**: 1908-1916.

MORA, M. A., SKILES, R., MCKINNEY, R., PAREDES, M., BUCKLER, D. & PAPOULIAS, D. K. D., 2002. Environmental contaminants in prey and tissues of the peregrine falcon in the Big Bend Region, Texas, USA. *Environ. Poll.*, 116: 169-176.

PARSONS, T. A. S., 2001. *Kenai National Wildlife Refuge Contaminant Assessment.* US Fish and Wildlife Service unpublished report. 1-123.

PETTY, J. D., JONES, S. B., HUCKINS, J. N., CRANOR, W. L., PARRIS, J. T., MCTAGUE, T. B. & BOYLE, T. P., 2000. – An approach for assessment of water quality using semipermeable membrane devices (SMDPs) as bioindicator tests. *Chemosphere*, **41**: 311-321.

SCHMITT, C. J., DETHLOFF, G. M., HINCK, J. E., BARTISH, T. M., BLAZER, V. S., COYLE, J. J., DENSLOW, N. D. & TILLITT, D. E., 2004. – *Biomonitoring of environmental status and trends (BEST) program: environmental contaminants and their effects on fish in the Rio Grande Basin*, US Geological Survey, Scientific Investigations Report 2004-5108: 1-117.

SEMLITSCH, R. D., SCOTT, D. E. & PECHMANN, J. H. K., 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology*, **69**: 184-192.

SMITH, D. C., 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. *Ecology*, **68**: 344-350.

SPARLING, D. W., FELLERS, G. M. & MCCONNELL, L. L., 2001. Pesticides and amphibian population declines in California, USA. *Environ. toxicol. Chem.*, **20**: 1591-1595.

STARNES, S. M., KENNEDY, C. A. & PETRANKA, J. W., 2000. Sensitivity of embryos of southern Appalachian amphibians to ambient solar UV-SS] radiation. *Conserv. Biol.*, **14**: 277-282.

STEARNS, S. C., 1992. *The evolution of life histories.* New York, New York, Oxford University Press: 1-249.

ZABIK, J. M. & SEIBER, J. N., 1993. Atmospheric transport of organophosphate pesticides from California's Central Valley to the Sierra Nevada Mountains. *J. environ. Qual.*, **22**: 80-90.

ZAGA, A., LITTLE, E. E., RABENI, C. F. & ELLERSIECK, M. R., 1998. Photoenhanced toxicity of a carbamate insecticide to early life stage anuran amphibians. *Environ. toxicol. Chem.*, **17**: 2543-2553.

Corresponding editor: C. Kenneth DODD, Jr

© ISSCA 2005

Source : MNHN, Paris

Alytes, 2005, **22** (3-4): 146-167.

Climate patterns as predictors of amphibian species richness and indicators of potential stress

William BATTAGLIN*, Lauren HAY**, Greg MCCABE**, Priya NANJAPPA*** & Alisa GALLANT****

* US Geological Survey, Box 25046, MS 415, Denver Federal Center, Lakewood, Colorado 80225, USA

** US Geological Survey, Box 25046, MS 412, Denver Federal Center, Lakewood, Colorado 80225, USA

*** US Geological Survey, 12100 Beech Forest Road, Laurel, Maryland 20708, USA

**** US Geological Survey, 47914 252nd Street, Sioux Falls, South Dakota 57198, USA

Amphibians occupy a range of habitats throughout the world, but species richness is greatest in regions with moist, warm climates. We modeled the statistical relations of anuran and urodele species richness with mean annual climate for the conterminous United States, and compared the strength of these relations at national and regional levels. Model variables were calculated for county and subcounty mapping units, and included 40-year (1960-1999) annual mean and mean annual climate statistics, mapping unit average elevation, mapping unit land area, and estimates of anuran and urodele species richness. Climate data were derived from more than 7,500 first-order and cooperative meteorological stations and were interpolated to the mapping units using multiple linear regression models. Anuran and urodele species richness were calculated from the United States Geological Survey's Amphibian Research and Monitoring Initiative (ARMI) National Atlas for Amphibian Distributions. The national multivariate linear regression (MLR) model of anuran species richness had an adjusted coefficient of determination (R^2) value of 0.64 and the national MLR model for urodele species richness had an R^2 value of 0.45. Stratifying the United States by coarse-resolution ecological regions provided models for anurans that ranged in R^2 values from 0.15 to 0.78. Regional models for urodeles had R^2 values ranging from 0.27 to 0.74. In general, regional models for anurans were more strongly influenced by temperature variables, whereas precipitation variables had a larger influence on urodele models.

INTRODUCTION

Amphibian populations appear to be declining worldwide (HOULAHAN et al., 2000, CAREY et al., 2001; YOUNG et al., 2004). A number of possible causes of decline have been proposed including changes in climate (e.g., POUNDS & CRUMP, 1994; DONNELLY & CRUMP, 1998, POUNDS et al., 1999), increased UV radiation (e.g., ALFORD & RICHARDS, 1999, BLAUSTEIN et al., 2003), habitat loss/fragmentation/alteration (e.g., FAHRIG et al., 1995,

Source : MNHN, Paris

DEMAYNADIER & HUNTER, 1998; KRZYSIK, 1998; COLLINS & STORFER, 2003), introduction of nonindigenous competitive species (e.g., HAYES & JENNINGS, 1986, ROSEN & SCHWALBE, 1995, FISHER & SHAFFER, 1996; KIESECKER & BLAUSTEIN, 1998; LAWLER et al., 1999), occurrence of contaminants (e.g., BERRILL et al., 1993, BONIN et al., 1997; DAVIDSON et al., 2002, HAYES et al., 2002), exposure to pathogens (e.g., LAURANCE et al., 1996, KIESECKER & BLAUSTEIN, 1999) and over-harvesting (KOONTZ, 1992; LANNOO, 1996). Many herpetologists believe that combinations of stresses are being placed on amphibian populations (GREEN, 1997; BRITSON & THRELKELD, 2000; COLLINS & STORFER, 2003; LANNOO et al., 2003; LITTLE et al., 2003).

There is widespread acknowledgment that the global climate is changing (HOUGHTON et al., 2001). Changes in land cover may also affect climate by altering the physical properties of the land surface (HAYDEN, 1998; PIELKE et al., 1999, 2002). Short- and long-term changes in climate have the potential to affect the ranges of individual amphibian species and hence species richness in any given locality (THOMAS et al., 2004). Climatic conditions not only directly stress amphibian populations (DODD, 1997, POUNDS et al., 1999, CORN & MUTHS, 2002), but also may influence their resistance to disease or their ability to withstand attacks by environmental pathogens (CAREY & ALEXANDER, 2003). Water availability, air temperature and relative humidity can influence amphibian breeding, development, foraging, mobility, calling, immune response and habitat availability (DONNELLY & CRUMP, 1998, GIBBS & BREISCH, 2001). Climate also can influence the spread of amphibian pathogens (DASZAK et al., 2003; JOHNSON & CHASE, 2004).

Amphibians have a long (~350 million years) history of survival under extremes in global climate (CAREY & ALEXANDER, 2003), yet their life histories (DUELLMAN, 1999*a*) suggest that individual amphibian populations may be vulnerable to short-term variations in climate. Amphibians occupy a range of habitats throughout the world, but species richness is greatest in regions with moist, warm climates (DUELLMAN, 1999*b*). Other natural factors that can affect amphibian species richness include historical lineages, barriers to migration, interspecies competition and the availability of food, shelter and breeding sites.

OBJECTIVES AND SCOPE

This research assesses the degree to which average climatic conditions in the conterminous United States over the last four decades explain historical patterns of amphibian species richness. The primary objectives of the research were to model the statistical relations of anuran and urodele species richness with mean annual climate for the conterminous United States, and to compare the strength of these relations at national and regional levels. Trends in climatic conditions during this period were also evaluated to determine if they might be leading towards more stressful conditions for amphibians (e.g., decreases in available breeding habitat, shortening of breeding season).

There were limitations or biases implicit in the datasets used for these analyses. First, the species occurrence records incorporated into the ARMI National Atlas for Amphibian Distributions were not associated with an explicit time period and are best described as an historic compilation of occurrence records. Second, the species richness estimates were

Source : MNHN, Paris

compared to climate statistics averaged for 1960-1999. Climate from this time period may not match longer-term averages or averages from other time periods. Third, the species richness estimates [http //www.mp2-pwrc usgs.gov/armiatlas/] were based on mapping units (counties/subcounties) that are not of uniform size, resulting in the potential for an inherent bias towards a larger number of species occurring in larger counties. Fourth, neither the weather stations nor the spatial variability of weather were uniformly distributed across the United States, so the quality of information varied from mapping unit to mapping unit Fifth, the mean elevation was used for each mapping unit, but the concept of average elevation may not be useful in mountainous areas. No attempt was made to account for the patchiness of the distribution of species within a county/subcounty (KIESTER, 1971), because such data do not exist for most of the United States, and no attempt was made to account for the effect of climate variation on species that prey upon amphibians or that amphibians consume. Despite these limitations, and because strong relationships between climate and ecosystem development are widely recognized (WALTER, 1973; FORMAN & GODRON, 1986; MONSERUD & LEEMANS, 1992), it was appropriate to expect that relations between climate and amphibian species richness would emerge from the analysis.

METHODS

SOURCE AND PROCESSING OF AMPHIBIAN DATA

Species richness estimates for anurans and urodeles were derived from the ARMI National Atlas for Amphibian Distributions (hereafter called "atlas") [http.//www.mp2-pwrc usgs.gov/armiatlas/], which uses a combination of counties and subcounties as a spatial framework for documenting the geographic occurrence of the nearly 300 species of amphibians currently recognized in the United States (LANNOO et al., 2005). Counties are used as mapping units for all but five Western states (Arizona, California, Nevada, Oregon, and Washington), for which subcounties are used to help overcome the wide disparity in county sizes across the nation. The atlas is a compilation of both current and historic records of amphibian occurrences, bounded by no explicit time period. The records are from peer-reviewed scientific literature, museum vouchers, state and regional herpetological atlases, and other confirmed and validated observations. Data sources vary by state and are not standardized in their geographic precision Thus, some records in the atlas may represent assumed presence, as from a range map, whereas other records represent vouchered specimens with specific location information. Because the atlas database incorporates a county/subcounty coding system that follows Federal Information Processing Standards, a geographic information system (GIS) was used to link species occurrence records with a digital map of county and subcounty polygons [http://www.census.gov/geo/www/cob/scale.html]. Species richness was calculated for anurans and urodeles by tallying the number of species recorded as occurring within each map unit.

Source : MNHN, Paris

SOURCE AND PROCESSING OF CLIMATE DATA

Estimates of 1960-1999 mean annual and annual mean climate statistics were calculated from approximately 7,500 National Weather Service first-order and cooperative temperature stations, and 11,500 National Weather Service first-order and cooperative precipitation stations. First-order stations are operated by professional staff and report a comprehensive array of weather variables each hour. Cooperative sites are more numerous, but generally only make once-daily observations of a few weather variables (e.g., minimum and maximum daily temperature and precipitation). These data were extracted from the National Climate Data Center Summary of the Day Dataset and have been quality controlled by the National Climate Data Center (EISCHEID et al., 2000; CLARK et al., 2004). Estimates of mean annual and annual mean climate statistics (tab. 1) for each county/subcounty were calculated using multiple linear regression (MLR) models. The MLR method was used to distribute the climate statistics (dependent variable) calculated at each station to each county/subcounty based on the "XYZ" value (longitude X, latitude Y, and mean elevation Z, respectively) of the county/subcounty polygon centroid (HAY et al., 2000, HAY & MCCABE, 2002; HAY & CLARK, 2003). The MLR equation [1] was developed for each dependent variable (climate statistic, "CS") using the independent XYZ variables from a set of National Weather Service climate stations.

$$CS = b_1 x + b_2 y + b_3 z + b_0 \quad [1]$$

The MLR equations were computed to determine the regression surface that described the spatial relations between the dependent CS and the independent XYZ variables. Equation [1] describes a plane in three-dimensional space with slopes b_1, b_2 and b_3 intersecting the CS axis at b_0. The best MLR equation for each CS did not always include all the independent variables.

To estimate the climate statistics for each county/subcounty (CNTY), the following procedures were followed: first, mean daily CS and corresponding mean XYZ values from a set of stations (STAMEAN) were used with the slopes of the MLR from equation [1] to estimate a unique y-intercept ($b_0$est, see equation [2]), and second, equation [3] was solved using the coefficients (b_1, b_2 and b_3) from equation [1], $b_0$est from equation [2], and the XYZ values of the CNTY.

$$b_0\text{est} = CS(\text{STAMEAN}) \quad (b_1\, x(\text{STAMEAN}) + b_2\, y(\text{STAMEAN}) + b_3\, z(\text{STAMEAN})) \quad [2]$$

$$CS(\text{CNTY}) = b_0\text{est} + b_1\, x(\text{CNTY}) + b_2\, y(\text{CNTY}) + b_3\, z(\text{CNTY}) \quad [3]$$

The set of stations comprising the STAMEAN in each calculation were chosen from the 20 closest stations to the CNTY. Outliers (i.e., stations determined to be too far away from the data site or residing in another physiographic region) were not used in the STAMEAN calculation. The same MLR equations are used but the time series of mean daily CS and their corresponding mean XYZ values are obtained from station data to estimate a unique $b_0$est. Thus, the slope of the MLR for the CS remained constant, but the y-intercept changes based on the mean CS and XYZ values.

Trends in climate were calculated by comparing, through regression analysis, the annual mean CS in each county/subcounty against time (year). When the annual mean CS values

Source : MNHN, Paris

Table 1. 1960-99 Mean annual climate statistics and other independent variables used for this study.

Climate statistic or other variable (and definition)	Unit	Variable name
Mean annual precipitation intensity (average for all days)	millimeters per day	PRE
Mean annual precipitation minus mean annual potential evapotranspiration (average for all years)	millimeters per year	PRE-PET
Mean annual minimum temperature (average for all days)	degrees Celsius	TMN
Mean annual mean temperature (average for all days)	degrees Celsius	TME
Mean annual maximum temperature (average for all days)	degrees Celsius	TMX
Mean annual number of wet days (days with measured precipitation)	days per year	WDAY
Mean annual number of dry days (days without measured precipitation)	days per year	DDAY
Mean annual number of cold days (days with minimum temperatures below 0°C)	days per year	CDAY
Mean annual number of hot days (days with maximum temperatures above 35°C)	days per year	HDAY
Mean annual solar radiation (average for all days)	Langley's per day	RAD
Mean annual total winter degree days ({$T_{base} - T_{ave}$} where T_{base} = 3°C and T_{ave} = {T_{max} + T_{min}} / 2, zero if negative)	dimensionless	WDD
Mean annual total summer degree days ({$T_{ave} - T_{base}$} where T_{base} = 3°C and T_{ave} = {T_{max} + T_{min}} / 2, zero if negative)	dimensionless	SDD
Mean elevation	meters	ELEV
County area (total land area of county)	square kilometers	AREA

were missing or zero, no trend was calculated and a zero trend value was assigned to the county/subcounty. Simulated CS in each county/subcounty for the years 1960 and 1999 were calculated using the trend regressions and the mean annual CS. Hence, the differences between the simulated CS values for the two years represent the magnitude of the trend over the 40 year time period and not the differences between any two years of actual CS data.

Source and processing of elevation and area data

Two additional variables used to augment the climate information for each county/subcounty were average elevation and total land area. Elevation data were obtained from the USGS National Elevation Dataset [http://edc.usgs.gov/products/elevation/ned.html] and were projected from geographic coordinates referenced to the World Geodetic Survey of 1984 to an Albers equal-area conic projection using a bilinear interpolation, 1000-meter cell resolution, and the following parameters: ellipsoid – World Geodetic Survey of 1984, 1st

Source : MNHN, Paris

standard parallel = 29.5°, 2nd standard parallel = 45.5°, central meridian = –96.0°, latitude of origin = 23.0°, and no false easting or northing. Average elevation was calculated as the mean of all cells within each mapping unit. Polygons for map units [http://www.census.gov/geo/www/cob/scale.html] were represented in an Albers equal-area conic projection using the same parameters as for the elevation data. Total mapping unit areas were determined from the county/subcounty polygons.

STATISTICAL METHODS

Multiple linear regression (MLR) models (HELSEL & HIRSCH, 1992) were developed using the SAS statistical software system (ANONYMOUS, 1990) to relate amphibian species richness to climate and location. Dependent and independent model variables were standardized by subtracting the respective mean and dividing by the respective standard deviation. Standardized variables have equal weights in regression models, and the resulting model coefficients are proportional to their explanatory power in the models. "Best" and "stepwise" SAS regression procedures were used to screen potential models; however, neither method prevents correlated independent variables from entering the models. Multicollinearity among independent variables, as indicated by variance inflation factor (VIF) values greater than 10, can cause MLR model coefficients to be unrealistic in sign or magnitude (HELSEL & HIRSCH, 1992). When an MLR model contained an independent variable with a VIF value greater than 3, the independent variable was not used.

Two sets of regression models were developed, one set for the entire conterminous United States (including one model for anurans and one for urodeles), and one set for each of 10 coarse-scale ecological regions (ANONYMOUS, 1997) (fig. 1). Because the primary objective of this research was to determine the degree to which climate explains patterns of amphibian species richness, model selection was manually supervised to favor climatic terms and prevent highly correlated independent variables from entering the same model. The adjusted coefficient of determination (R^2) and root mean square error (*RMSE*) statistics were used to evaluate the predictive skill of the models for a particular region or the nation (ANONYMOUS, 1990; HELSEL & HIRSCH, 1992). The residuals between model and atlas estimates of species richness are used to compare the predictive capabilities of the national and regional models. Box plots are used to show the distributions of these residuals. The box plots show high and low outliers as circles. The central box extends from the 25th to 75th percentile of the data, and the box whiskers extend to the 5th and 95th percentiles.

RESULTS

NATIONAL REGRESSION MODELS

Anuran species richness ranged from a maximum of 26 to a minimum of 1 (fig. 2a). The R^2 for the national anuran model (fig. 3a) was 0.64 (tab. 2), with an *RMSE* of 3.07 species. Mean annual temperature and mean annual precipitation (fig. 1b) accounted for the largest

Source : MNHN, Paris

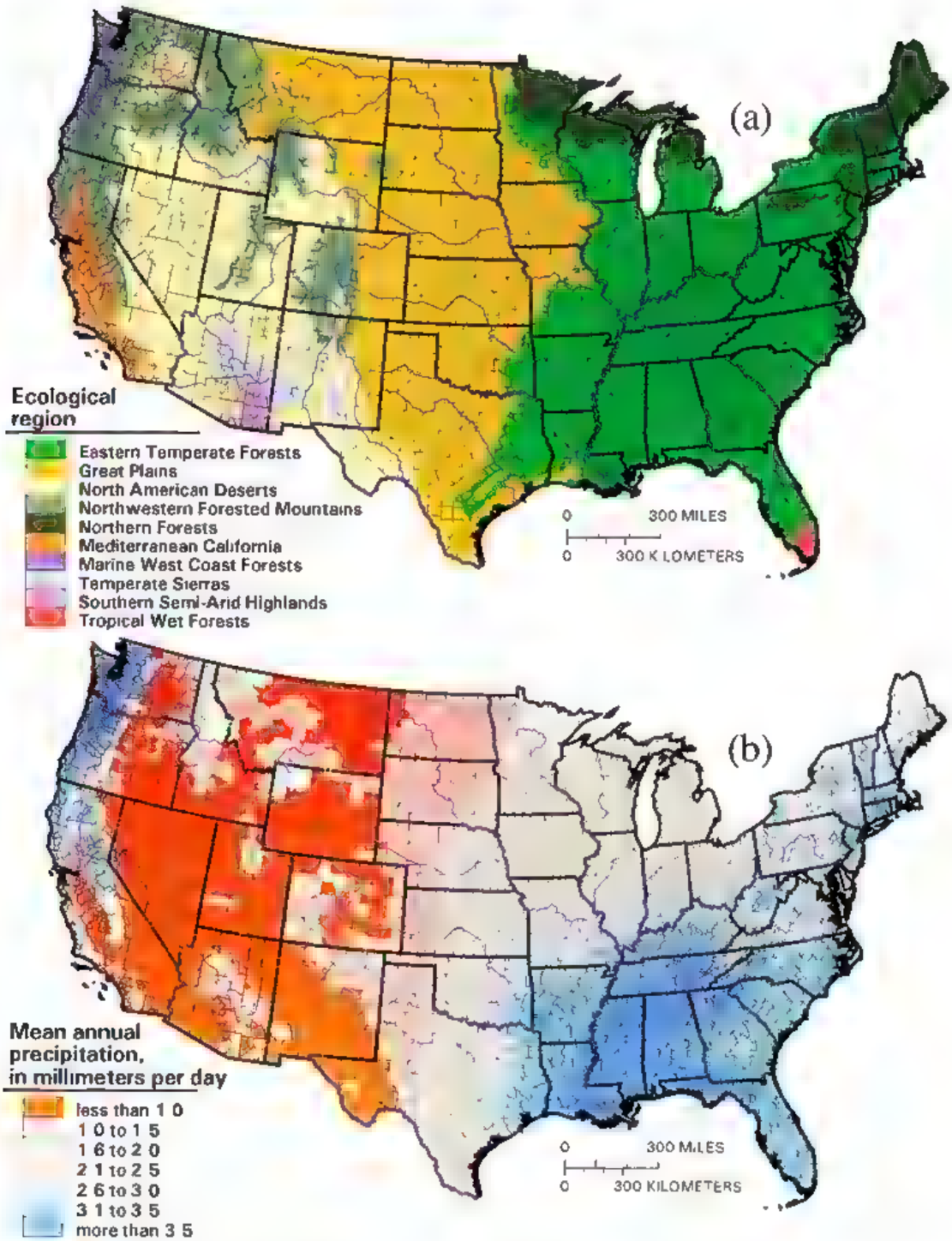


Fig. 1 Maps showing in the conterminous United States (a) coarse-level ecological regions and State, county and subcounty boundaries, and (b) 1960-1999 mean annual precipitation

Source : MNHN, Paris

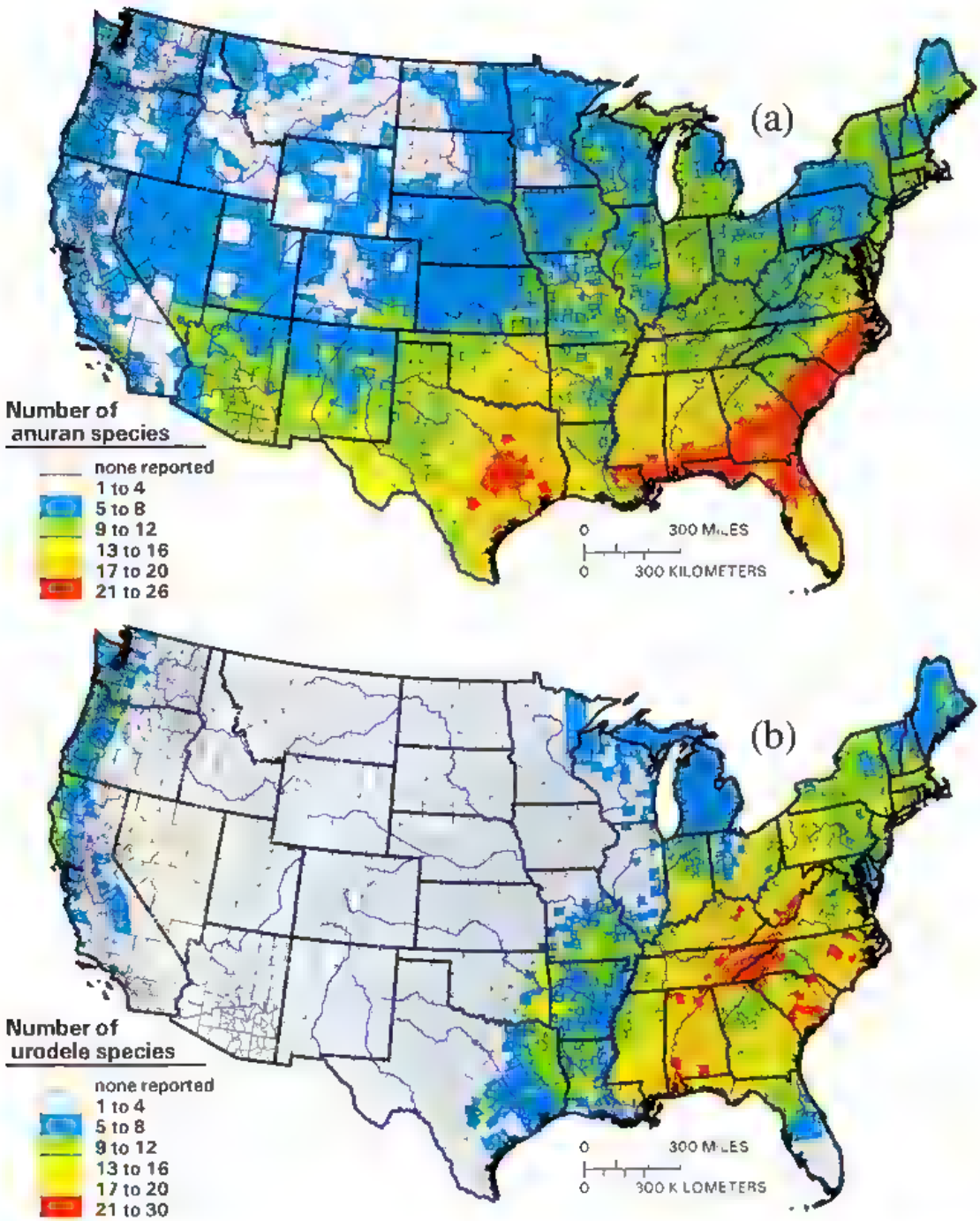


Fig. 2. Maps showing in the conterminous United States (a) anuran species richness and (b) urodele species richness, both from the Amphibian Research and Monitoring Initiative (ARMI) National Atlas for Amphibian Distributions.

Source : MNHN, Paris

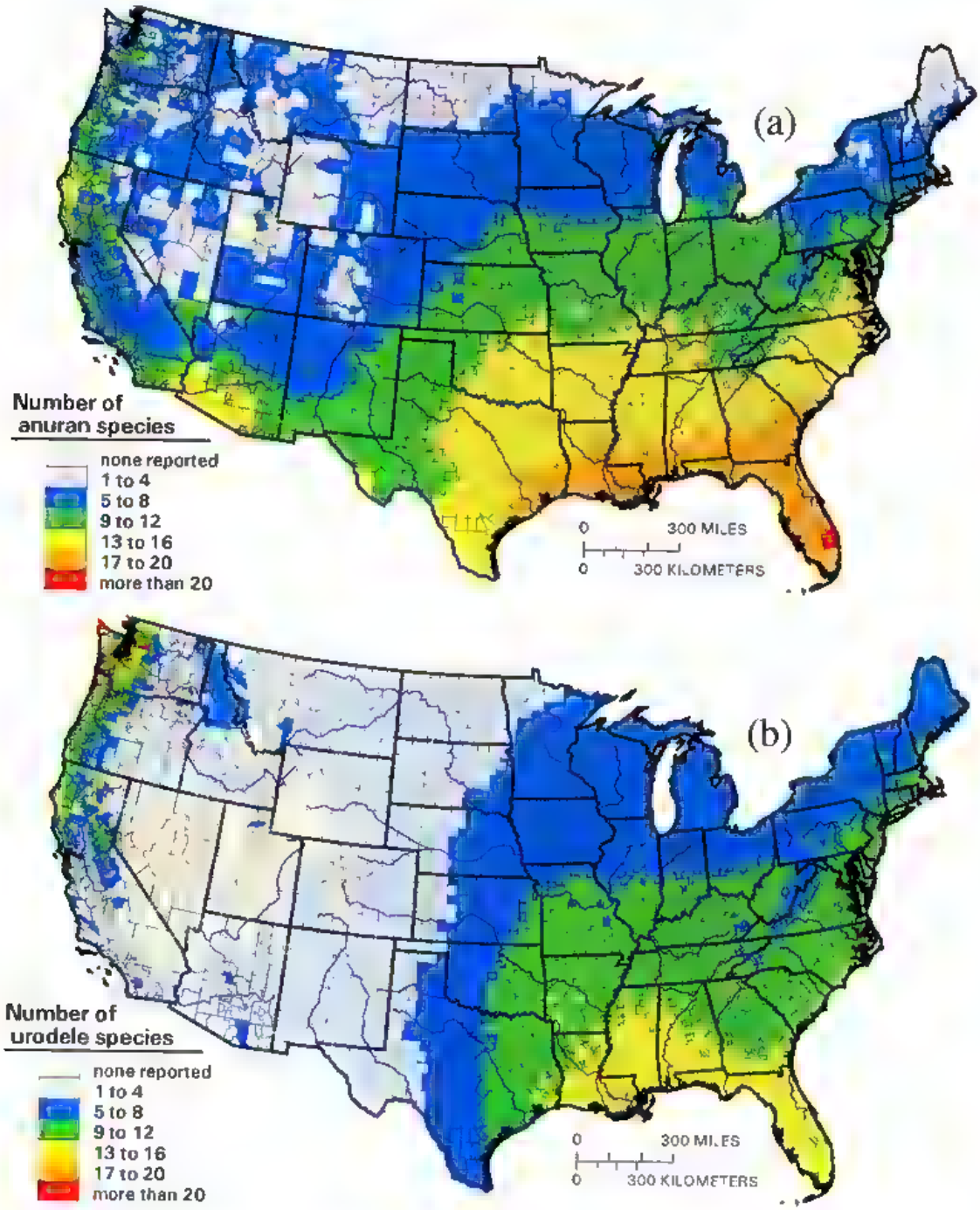


Fig. 3 – Maps showing in the conterminous United States the national regression model estimates of (a) anuran species richness and (b) urodele species richness.

Source : MNHN, Paris

Tab 2. Best-fitting national and ecological region standardized regression models of amphibian species richness and adjusted coefficient of determination (R^2) NA, North American; NW, Northwestern See tab. 1 for other abbreviations

National or ecological Region	Regression model	R^2
Anurans		
National	0 57*PRE + 0 56*TME - 0 49*PRE-PET + 0.07*ELEV	0.64
Eastern Temperate Forests	0 71*TMX – 0.21*ELEV + 0.10*WDAY + 0 07*AREA	0 63
Great Plains	0 78*TME + 0 18*PRE + 0 08*AREA – 0 07*ELEV	0.78
NA Deserts	0 79*TMX + 0 36*PRE + 0 22*AREA + 0.16*ELEV	0 47
NW Forested Mountains	-0 31*WDD - 0.27*ELEV + 0 25*AREA	0 23
Northern Forests	-0 35*ELEV + 0 34*CDAY + 0 33*PRE	0 15
Mediterranean California	0 45*ELEV – 0 41*CDAY + 0.39*RAD + 0 21*PRE	0 34
Marine West Coast Forests	0 34*TME + 0.22*ELEV + 0 14*AREA	0 25
Temperate Sierras	No statistically significant model.	-
Southern Semi-Arid Highlands	0 80*ELEV + 0 68*RAD – 0 68*CDAY	0 68
Tropical Wet Forests	Too few mapping units (5) to develop a model.	-
Urodeles		
National	0.70*PRE - 0 24*PRE-PET + 0.11*TME - 0.05*ELEV	0 45
Eastern Temperate Forests	-0 65*WDD + 0 32*ELEV + 0 29*WDAY + 0 15*PRE-PET	0 50
Great Plains	0 55*PRE + 0.20* TME + 0 16*PRE-PET + 0 15*AREA	0 49
NA Deserts	-0 57*ELEV + 0 37*PRE + 0 20*AREA – 0 19*TME	0.27
NW Forested Mountains	0 55*PRE + 0 20*TME – 0 18*ELEV	0.60
Northern Forests	-0 67*WDD + 0 35*ELEV + 0 33*PRE	0 74
Mediterranean California	0 47*PRE – 0 43*WDD 0 34*TMX + 0 26*ELEV	0 50
Marine West Coast Forests	0 33*PRE + 0 31*TME + 0 23*ELEV + 0 22*RAD	0 40
Temperate Sierras	No statistically significant model.	-
Southern Semi-Arid Highlands	No statistically significant model.	-
Tropical Wet Forests	Too few mapping units (5) to develop a model	-

proportion of the variation (because they have the largest model coefficients, tab 2), and were both positively associated with species richness Mean annual precipitation minus mean annual potential evapotranspiration also accounted for a substantial proportion of the variation and was inversely associated with species richness. Mean mapping unit elevation accounted for a small proportion of the variation and was positively associated with anuran species richness The national regression model overestimated anuran species richness along the Mississippi embayment and in parts of California, Florida, and Oregon The model underestimated anuran species richness along the Atlantic coastal plain and in parts of Maine and Texas (fig 2a, 3a).

Urodele species richness ranged from a maximum of 30 to a minimum of 0 (fig. 2) The R^2 for the national urodele model (fig 3b) was 0 45, with an *RMSE* of 4 54 species. The

Source : MNHN, Paris

national urodele model (tab. 2) used the same CS as the national anuran model, but the coefficient values were appreciably different. Mean annual precipitation accounted for the largest proportion of the variation and was positively associated with species richness. Mean annual precipitation minus mean annual potential evapotranspiration accounted for a smaller proportion of the variation and was inversely associated with species richness. The national regressions model overestimated urodele species richness in the central United States and in parts of Florida and Washington; and underestimated species richness in most of the Eastern United States except for Florida and Maine (fig. 2b, 3b).

Regional regression models

Separate regression models were developed for each coarse-resolution ecological region (fig. 1a) to evaluate regional differences in the strength of climate as a predictor of amphibian species richness. No models were developed for the Tropical Wet Forest ecological region, which was predominant only in five mapping units, and represented less than 0.3 % of the conterminous United States. The mean of anuran and urodele species richness for these five mapping units was used in place of a model.

Eastern Temperate Forests

The Eastern Temperate Forests ecological region was predominant in 1,789 mapping units, representing 31.8 % of the conterminous United States (fig. 1a). Anuran species richness in this ecological region ranged from 2 to 26, and urodele species richness ranged from 1 to 30. The R^2 for the Eastern Temperate Forest ecological region anuran model (fig. 4a) was 0.63, and the *RMSE* was 2.91 species. Mean annual maximum temperature accounted for the largest proportion of the variation and was positively associated with species richness. The Eastern Temperate Forest model overestimated anuran species richness in parts of Arkansas, Florida and Louisiana, and underestimated anuran species richness along the Atlantic Coastal Plain and in parts of Alabama and Indiana. The residuals (model estimate minus atlas estimate) for the Eastern Temperate Forest anuran model were much smaller than the residuals between national model and atlas estimates of anuran species richness in those same mapping units (fig. 5a; gray box plots are residuals from regional models and black box plots are residuals from national model in the same mapping units). The R^2 for the Eastern Temperate Forest ecological region urodele model (fig. 4b) was 0.50, and the *RMSE* was 3.78 species. The total of mean annual winter degree days accounted for the largest proportion of the variation, and was inversely associated with species richness. The Eastern Temperate Forest model overestimated urodele species richness in parts of Arkansas, Florida, Illinois, Louisiana and Texas, and underestimated urodele species richness along the Eastern coastal and inland plains and in parts of Alabama, Indiana and Kentucky. The residuals for the Eastern Temperate Forest urodele model were smaller than the residuals between the national model and atlas estimates of urodele species richness (fig. 5b).

Source : MNHN, Paris

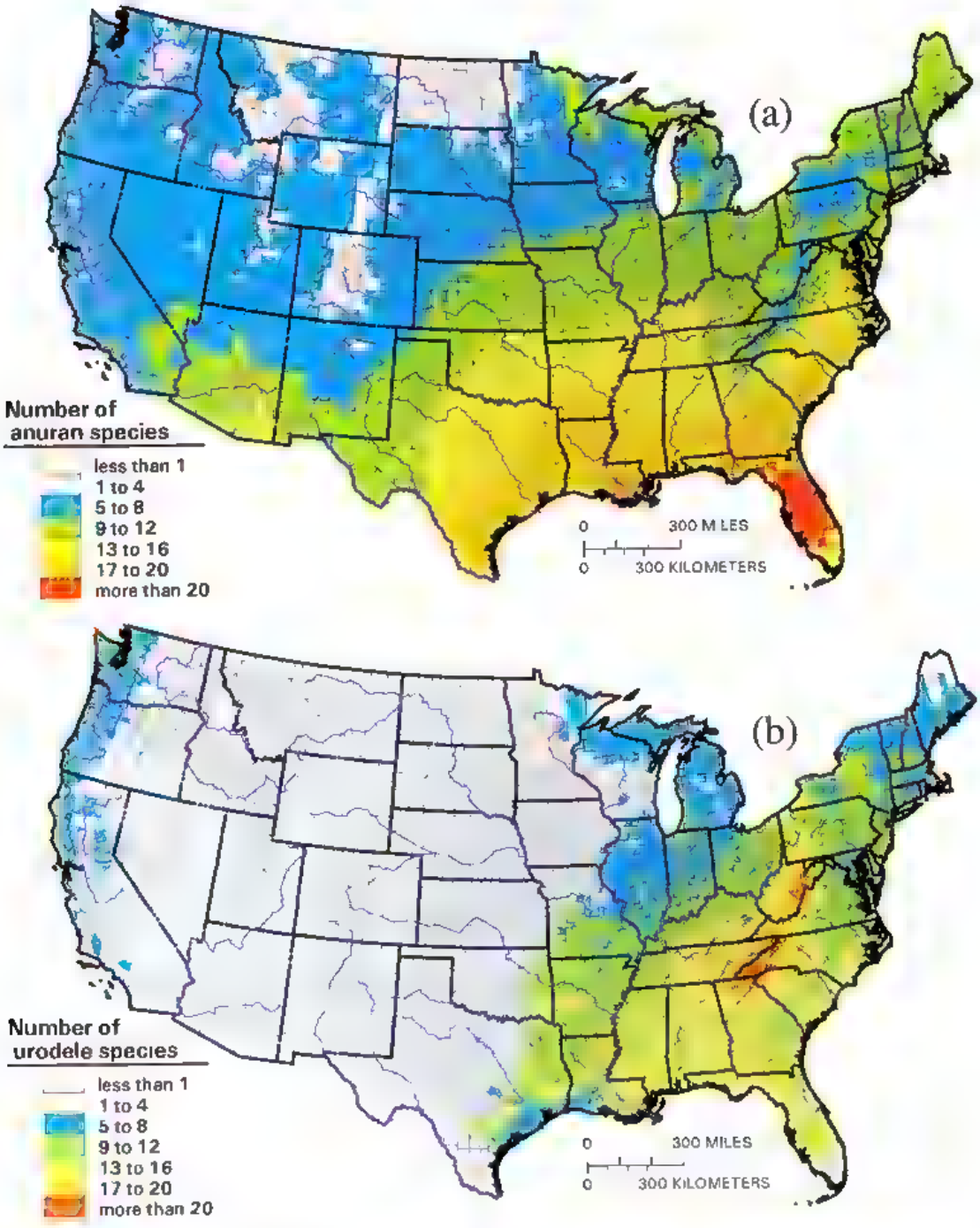


Fig. 4. Maps showing in the conterminous United States a compilation of regional regression models estimates of (a) anuran species richness and (b) urodele species richness.

Source : MNHN, Paris

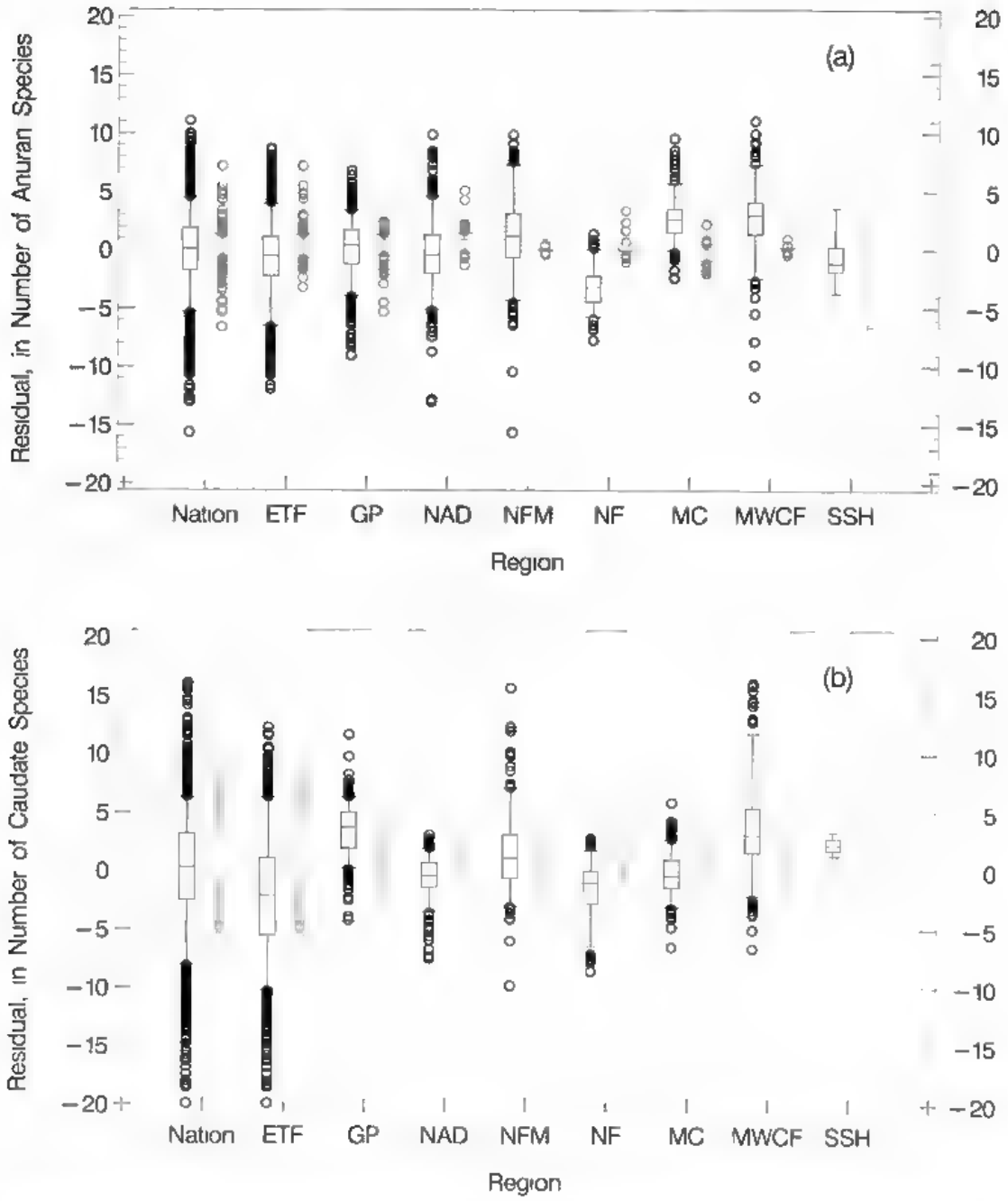


Fig 5 Box plots showing residuals (in species) between national regression model and atlas (black) and regional regressions models and atlas (gray) estimates of (a) anuran species richness and (b) urodele species richness ETF, Eastern Temperate Forest, GP, Great Plains, NAD, North American Deserts, NFM, Northwestern Forestal Mountains; NF, Northern Forests; MC, Mediterranean California, MWCF, Marine West Coast Forests, SSH, Southern Semi-Arid Highlands.

Source : MNHN, Paris

GREAT PLAINS

The Great Plains ecological region was predominant in 837 mapping units, representing 28.9 % of the conterminous United States (fig. 1a). Anuran species richness in this ecological region ranged from 1 to 23, and urodele species richness ranged from 0 to 13. The R^2 for the Great Plains ecological region anuran model (fig. 4a) was 0.78, and the *RMSE* was 2.12 species. Mean annual temperature accounted for the largest proportion of the variation and was positively associated with species richness. The Great Plains model overestimated anuran species richness in parts of Kansas, Missouri and Nebraska; and underestimated anuran species richness in parts of North Dakota, Oklahoma and Texas. The residuals for the Great Plains anuran model were much smaller than the residuals between the national model and atlas estimates of anuran species richness (fig. 5a). The R^2 for the Great Plains ecological region urodele model was 0.49, and the *RMSE* was 1.10 species. mean annual precipitation accounted for the largest proportion of the variation and was positively associated with species richness. The Great Plains model overestimated urodele species richness in parts of Iowa, Kansas and Oklahoma, and underestimated urodele species richness in parts of North Dakota and Texas. The residuals for the Great Plains model were much smaller than the residuals between the national model and atlas estimates of urodele species richness (fig. 5b).

NORTH AMERICAN DESERTS

The North American Deserts ecological region was predominant in 349 mapping units representing 19.8 % of the conterminous United States (fig. 1a). Anuran species richness in this ecological region ranged from 1 to 16, and urodele species richness ranged from 0 to 4. The R^2 for the North American Deserts ecological region anuran model (fig. 4a) was 0.47, and the *RMSE* was 2.05 species. Mean annual maximum temperature accounted for the largest proportion of the variation and was positively associated with species richness. The North American Deserts model overestimated anuran species richness in parts of California and Utah, and underestimated anuran species richness in parts of Arizona and Texas. The residuals for the North American Deserts anuran model were much smaller than the residuals between the national model and atlas estimates of anuran species richness (fig. 5a). The R^2 for the North American Deserts ecological region urodele model (fig. 4b) was 0.27, and *RMSE* was 0.65 species. Mean mapping unit elevation accounted for the largest proportion of the variation and was inversely associated with species richness. The North American Deserts model overestimated urodele species richness in parts of California and Nevada, and underestimated urodele species richness in parts of California. The residuals for the North American Deserts urodele model were smaller than the residuals between the national model and atlas estimates of urodele species richness (fig. 5b).

NORTHWESTERN FORESTED MOUNTAINS

The Northwestern Forested Mountains ecological region was predominant in 289 mapping units, representing 9.1 % of the conterminous United States (fig. 1a). Anuran species

Source : MNHN, Paris

richness in this ecological region ranged from 1 to 8, and urodele species richness ranged from 0 to 12. The R^2 for the Northwestern Forested Mountains ecological region anuran model (fig. 4a) was 0.23, and the *RMSE* was 1.20 species. The total of mean annual winter degree days accounted for the largest proportion of the variation and was inversely associated with species richness. The Northwestern Forested Mountains model overestimated anuran species richness in parts of Colorado and Idaho, and underestimated anuran species richness in parts of Oregon. The residuals for the Northwestern Forested Mountains anuran model were much smaller than the residuals between the national model and atlas estimates of anuran species richness (fig. 5a) The R^2 for the Northwestern Forested Mountains ecological region urodele model (fig. 4b) was 0.60, and the *RMSE* was 1.77 species. mean annual precipitation accounted for the largest proportion of the variation and was positively associated with species richness. The Northwestern Forested Mountains model overestimated urodele species richness in parts of Washington, and underestimated urodele species richness in parts of Colorado and Oregon. The residuals for the Northwestern Forested Mountains urodele model were much smaller than the residuals between the national model and atlas estimates of urodele species richness (fig. 5b).

Northern Forests

The Northern Forests ecological region was predominant in 134 mapping units, representing 5.2 % of the conterminous United States (fig. 1a) Anuran species richness in this ecological region ranged from 5 to 10, and urodele species richness ranged from 1 to 15. The R^2 for the Northern Forests ecological region anuran model was 0.15, and the *RMSE* was 0 92 species. Mean mapping unit elevation accounted for the largest proportion of the variation and was inversely associated with species richness. The residuals for the Northern Forests anuran model were much smaller than the residuals between the national model and atlas estimates of anuran species richness (fig. 5a). The R^2 for the Northern Forests ecological region urodele model was 0.74, and the *RMSE* was 1.60 species. The total of mean annual winter degree days accounted for the largest proportion of the variation and was inversely associated with species richness. The residuals for the Northern Forests urodele model were slightly smaller than the residuals between the national model and atlas estimates of urodele species richness (fig. 5b).

Mediterranean California

The Mediterranean California ecological region was predominant in 277 mapping units, representing 2 1 % of the conterminous United States (fig. 1a) Anuran species richness in this ecological region ranged from 2 to 9, and urodele species richness ranged from 0 to 10 The R^2 for the Mediterranean California ecological region anuran model was 0 34, and the *RMSE* was 1 04 species. Mean mapping unit elevation accounted for the largest proportion of the variation and was positively associated with species richness. The residuals for the Mediterranean California anuran model were much smaller than the residuals between the national model and atlas estimates of anuran species richness (fig. 5a) The R^2 for the Mediterranean California ecological region urodele model was 0 50, and the *RMSE* was 1.46 species. Mean

Source : MNHN, Paris

annual precipitation accounted for the largest proportion of the variation and was positively associated with species richness. The residuals for the Mediterranean California urodele model were smaller than the residuals between the national model and atlas estimates of urodele species richness (fig. 5b).

MARINE WEST COAST FORESTS

The Marine West Coast Forests ecological region was predominant in 219 mapping units, representing 1.1 % of the conterminous United States (fig 1a) Anuran species richness in this ecological region ranged from 3 to 6, and urodele species richness ranged from 2 to 10. The R^2 for the Marine West Coast Forests ecological region anuran model was 0.25, and the *RMSE* was 0 67 species. Mean annual temperature accounted for the largest proportion of the variation and was positively associated with species richness. The residuals for the Marine West Coast Forests anuran model were much smaller than the residuals between the national model and atlas estimates of anuran species richness (fig. 5a). The R^2 for the Marine West Coast Forests ecological region urodele model was 0 40, and the *RMSE* was 1 55 species. Mean annual precipitation accounted for the largest proportion of the variation and was positively associated with species richness. The residuals for the Marine West Coast Forests urodele model were much smaller than the residuals between the national model and atlas estimates of urodele species richness (fig. 5b).

TEMPERATE SIERRAS

The Temperate Sierras ecological region was predominant in 18 mapping units representing 1 1 % of the conterminous United States (fig 1a). Anuran species richness in this ecological region ranged from 8 to 14, and urodele species richness was always 1. No statistically significant model of anuran species richness could be developed from the available independent variables (tab. 2). No model of urodele species richness was attempted since there was no variation in the dependent variable.

SOUTHERN SEMI-ARID HIGHLANDS

The Southern Semi-Arid Highlands ecological region was predominant in 17 mapping units representing 0 6 % of the conterminous United States (fig 1a) Anuran species richness in this ecological region ranged from 9 to 15, and urodele species richness was either 0 or 1 The R^2 for the Southern Semi-Arid Highlands ecological region anuran model was 0 68, and the *RMSE* was 0.88 species. Mean mapping unit elevation accounted for the largest proportion of the variation and was positively associated with species richness. The residuals for the Southern Semi-Arid Highlands anuran model were slightly larger than the residuals between the national model and atlas estimates of anuran species richness (fig 5a) No statistically significant model of urodele species richness could be developed from the available independent variables due to the limited variation in the dependent variable.

Source : MNHN, Paris

Climate trends

Amphibian species richness was strongly associated with several of the mean annual climate variables, and mean annual precipitation and mean annual temperature were statistically significant variables in 12 and 8 models, respectively (tab. 2). Increasing trends in annual mean temperature and precipitation were prevalent across much of the conterminous United States between 1960 and 1999 (fig. 6). Exceptions include decreasing mean annual temperature in parts of the Great Plains ecological region, and decreasing mean annual precipitation in the southeastern part of the Eastern Temperate Forests ecological region.

Discussion

At the national level, the model for anurans performed better than that for urodeles (fig. 5). Both models included mean annual precipitation as a strong variable for predicting patterns of richness. Kiester (1971) and Duellman & Sweet (1999) previously noted a strong correlation in the conterminous United States between amphibian species richness and mean annual rainfall. Partitioning the country by coarse-resolution ecological regions resulted in improved models for both anurans and urodeles. The residuals (model estimate minus atlas estimate) for the compilation of regional anuran and urodele models were much smaller than the residuals between national model and atlas estimates of anuran and urodele species richness in all mapping units (fig. 5). In several cases the R^2 for the regional models were less than that of the national model, but the residuals were also smaller. In general, temperature variables (mean annual mean and mean annual maximum) figured more strongly in anuran models, whereas precipitation (mean annual precipitation intensity) had greater explanatory value in urodele models. This makes sense from the perspective that there is no urodele counterpart to toads, hence, anurans are less restricted by arid conditions than are urodeles.

In general, trends in climate during 1960-1999 were toward wetter, warmer conditions for most of the conterminous United States. This could have provided more surface moisture availability for breeding habitat, and air and soil temperatures more amenable to regulating amphibian body temperatures throughout the year. Trends toward drier conditions in part of the southeastern United States and southwest Oregon may have resulted in reduced availability of breeding habitat in those areas.

This effort to model the relations between anuran and urodele species richness and mean annual climate in the United States capitalized on the strong dependence of amphibians on their external environment for internal hydrothermal regulation. A limitation of the approach was that it assumed that the climate experienced by amphibians was reflected by long-term climate statistics summarized at the county/subcounty level. In fact, amphibians interact with climate at multiple scales, and alter their behaviors in concert with microhabitat features (sun flecks, burrows, duff, vegetation cover, wetlands, etc.) to modify the effects of the broader-scale conditions. Therefore, the conditions represented by the data in this study likely addressed only the broadest effects of climate. For this reason, the statistical models presented

Source : MNHN, Paris

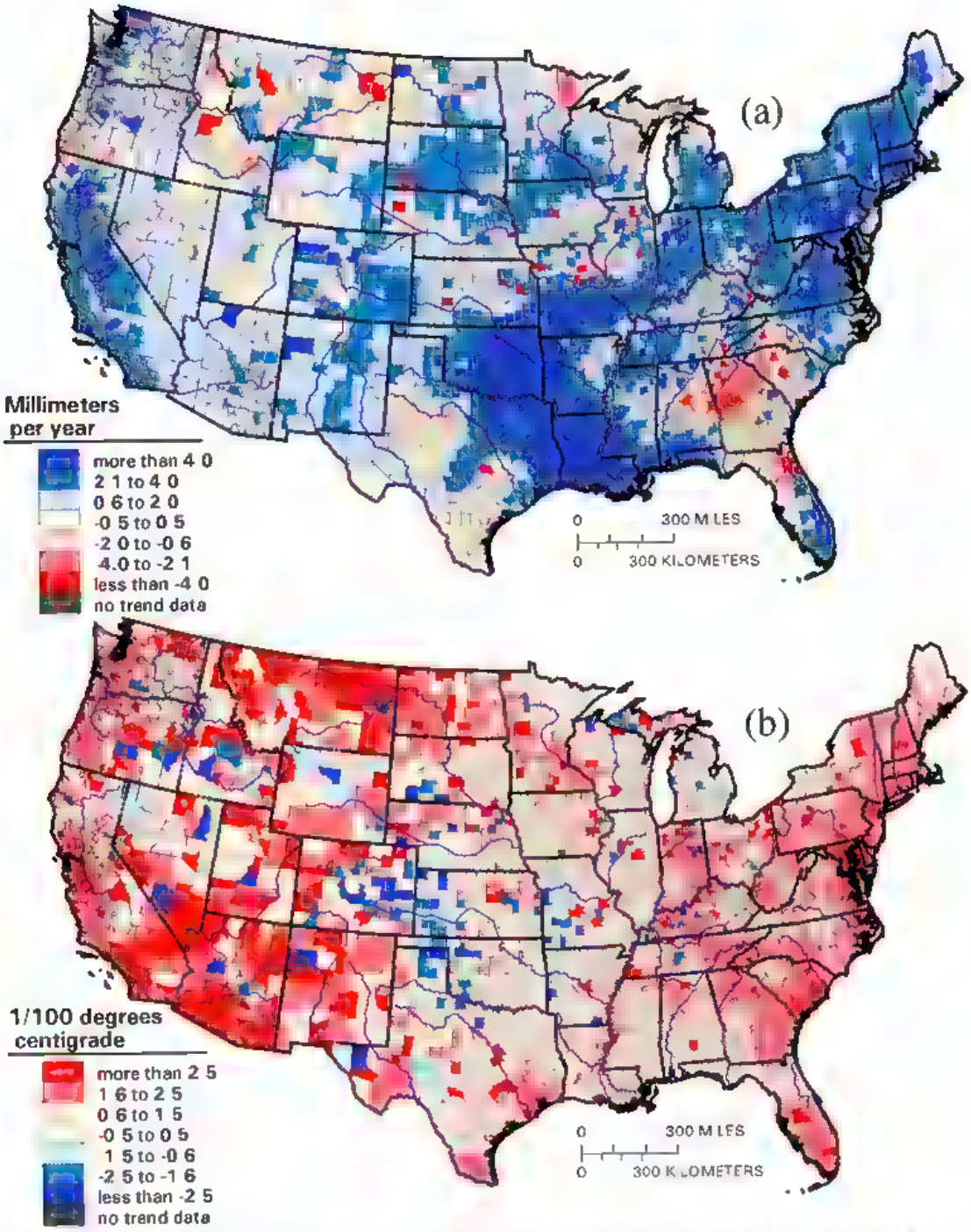


Fig 6 Maps showing in the conterminous United States the trend for 1960 1999 in (a) annual mean precipitation and (b) annual mean temperature

Source : MNHN, Paris

here were aimed at the general level of anuran and urodele richness, and were not aimed at predicting the fate of particular species.

Other explanatory variables may improve the ability to explain patterns of amphibian richness. For example, seasonal climate statistics may be more informative than annual statistics for certain measures, and additional landscape factors (e.g., current and historic land cover/use, hydrology, glaciation) and information such as evolutionary lineage could be very useful. Additionally, models could be developed at the family level, or for groups of species having similar life history or developmental characteristics.

The coarse-level ecological regions used for this study were somewhat problematic. Highly discontinuous mountain regions in the West often did not align well with county/subcounty units, so not all discontinuous portions of these regions were represented in the models. The largest regions (Eastern Temperate Forests, Great Plains and North American Deserts) included a lot of variation in temperature and moisture gradients. A finer level of regionalization (Level II regions defined in ANONYMOUS, 1997) may have been more appropriate, as it would have subdivided the largest regions, while leaving the smaller regions intact, however, the number of map units per region may have been insufficient for developing models for several of the regions at this level.

RÉSUMÉ

Les amphibiens occupent une grande diversité d'habitats sur la planète, mais leur richesse spécifique est plus élevée dans les régions aux climats humides et chauds. Nous avons modélisé les relations statistiques entre la richesse spécifique en anoures et urodèles et le climat annuel des Etats Unis continentaux, et compare ces relations aux niveaux national et régional. Les variables modélisées ont été calculées pour des unités cartographiques correspondant aux contés ou aux sous-contés, et se sont appuyées sur des statistiques climatiques annuelles moyennes recueillies sur une période de 40 années (1960-1999), l'altitude moyenne et la surface des unités cartographiques, et des estimations de la richesse spécifique en anoures et urodeles. Les données climatiques ont éte obtenues à partir de plus de 7500 stations météorologiques et ont eté incorporées dans les données concernant les unités cartographiques au moyen de modèles de régression linéaire multiple. Les richesses specifiques en anoures et urodèles ont été calculées à partir de l'atlas national de distribution des amphibiens préparé par l'Amphibian Research and Monitoring Initiative (ARMI) de l'United States Geological Survey. Le modèle de régression lineaire multivariée (MLR) national pour la richesse spécifique en anoures a un coefficient de détermination ajuste (R^2) de 0,64 et celui concernant les urodèles un R^2 de 0,45. Lorsque les Etats Unis sont divisés en regions ecologiques grossieres, on obtient des modèles pour les anoures dont les R^2 se répartissent entre 0,15 et 0,78 pour les anoures, et entre 0,27 et 0,74 pour les urodèles. En général, les modèles régionaux pour les anoures se sont avéres plus fortement influencés par des variables de temperature, tandis que les variables liées à la precipitation avaient plus d'influence sur les modèles pour les urodeles.

Source : MNHN, Paris

ACKNOWLEDGMENTS

The United States Geological Survey's Toxics Program and Amphibian Research and Monitoring Initiative provided funding to support this study. We thank S. Char for support with GIS analysis. We are grateful to B. Moring, USGS, Texas, and B. Klaver, USGS, South Dakota, and two anonymous reviewers for comments on earlier versions of this manuscript.

LITERATURE CITED

ANONYMOUS [SAS INSTITUTE]. 1990. – *SAS/STAT user's guide*, Version 6, 4th edition, Cary, North Carolina, 1,686 pp.

ANONYMOUS [Commission for Environmental Cooperation]. 1997. – Ecological regions of North America. Towards a common perspective. Montreal, Canada, CEC: 1-71.

ALFORD, R. A. & RICHARDS, S. J., 1999. – Global amphibian declines: a problem in applied ecology. *Ann. Rev. Ecol. Syst.*, **30**: 133-165.

BERRILL, M., BERTRAM, S., WILSON, A., LOUIS, S., BRIGHAM, D. & STROMBERG, C., 1993. – Lethal and sublethal impacts of pyrethroid insecticides on amphibian embryos and tadpoles. *Environ. Toxicol. Chem.*, **12**: 525-539.

BLAUSTEIN, A. R., ROMANSIC, J. M., KIESECKER, J. M. & HATCH, A. C., 2003. – Ultraviolet radiation, toxic chemicals and amphibian population declines. *Diver. & Distrib.*, **9**: 123-140.

BONIN, J., OLELLET, M., RODRIGUE, J., DESGRANGES, J.-L., GAGNE, F., SHARBEL, T. F. & LOWCOCK, L. A., 1997. – Measuring the health of frogs in agricultural habitats subjected to pesticides. *In* D. M. GREEN (ed.), *Amphibians in decline: Canadian studies of a global problem. Herpetological Conservation*, St. Louis, Missouri, Society for the Study of Amphibians and Reptiles, **1**: 246-257.

BRITSON, C. A. & THRELKELD, S. T., 2000. – Interactive effects of anthropogenic, environmental, and biotic stressors on multiple endpoints in *Hyla chrysoscelis*. *J. Iowa Acad. Sci.*, **107**: 61-66.

CAREY, C. & ALEXANDER, M. A., 2003. – Climate change and amphibian declines: is there a link? *Diver. & Distrib.*, **9**: 111-121.

CAREY, C., HEYER, W. R., WILKINSON, J., ALFORD, R. A., ARNTZEN, J. W., HALLIDAY, T., HUNGERFORD, L., LIPS, K. R., MIDDLETON, E. M., ORCHARD, S. A. & RAND, A. S., 2001. – Amphibian declines and environmental change: use of remote-sensing data to identify environmental correlates. *Conserv. Biol.*, **15**: 2001.

CLARK, M., HAY, L., RAJOGOPALAN, B. & WILBY, R., 2004. – The Schaake shuffle: a method for reconstructing space-time variability in forecasted precipitation and temperature fields. *J. Hydrometeorol.*, **5**: 243-262.

COLLINS, J. P. & STORFER, A., 2003. – Global amphibian declines: sorting the hypotheses. *Diver. & Distrib.*, **9**: 89-98.

CORN, P. S. & MUTHS, E., 2002. – Variable breeding phenology affects the exposure of amphibian embryos to ultraviolet radiation. *Ecology*, **83**: 2958-2963.

DASZAK, P., CUNNINGHAM, A. A. & HYATT, A. D., 2003. – Infectious disease and amphibian population declines. *Diver. & Distrib.*, **9**: 141-150.

DAVIDSON, C., SHAFFER, H. B. & JENNINGS, M. R., 2002. – Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. *Conserv. Biol.*, **16**: 1588-1601.

DEMAYNADIER, P. G. & HUNTER, M. L., Jr., 1998. – Effects of silvicultural edges on the distribution and abundance of amphibians in Maine. *Conserv. Biol.*, **12**: 340-352.

DODD, C. K., Jr., 1997. – Imperiled amphibians: a historical perspective. *In* G. BENZ & D. COLLINS (ed.), *Aquatic fauna in peril: the southeastern perspective*, Special Publication **1**, Decatur, Georgia, Southeast Aquatic Research Institute, Lenz Design & Communications: 165-200.

Source : MNHN, Paris

DONNELLY, M. A. & CRUMP, M. L., 1998. – Potential effects of climate change on two neotropical amphibian assemblages. *Clim. Change*, **39**: 541-561.

DUELLMAN, W. E., 1999*a*. – *Patterns of distribution of amphibians: a global perspective*. Baltimore, Maryland, The John Hopkins University Press: 1-633.

---- 1999*b*. – Global distribution of amphibians: patterns, conservation, and future challenges. *In* DUELLMAN (1999*a*): 1-30.

DUELLMAN, W. E. & SWEET, S. S., 1999. – Distribution patterns of amphibians in the nearctic region of North America. *In* DUELLMAN (1999*a*): 31-110.

FISCHEID, J. K., PASTERIS, P. A., DIAZ, H. F., PLANTICO, M. S. & LOTT, N. J., 2000. – Creating a serially complete, national daily time series of temperature and precipitation for the western United States. *J. appl. Meteorol.*, **39**: 1580-1591.

FAHRIG, L., PEDLAR, J. H., POPE, S. E., TAYLOR, P. D. & WEGNER, J. F., 1995. – Effect of road traffic on amphibian density. *Biol. Conserv.*, **73**: 177-182.

FISHER, R. N. & SHAFFER, H. B., 1996. – The decline of amphibians in California's Great Central Valley. *Conserv. Biol.*, **10**: 1387-1397.

FORMAN, R. T. T. & GODRON, M., 1986. – *Landscape ecology*. New York, New York, John Wiley & Sons: 1-620.

GIBBS, J. P. & BREISCH, A. R., 2001. – Climate warming and calling phenology of frogs near Ithaca, New York, 1990-1999. *Conserv. Biol.*, **15**: 1175-1178.

GREEN, D. M., 1997. – Perspectives on amphibian population declines: defining the problem and searching for answers. *In* D. M. GREEN (ed.), *Amphibians in decline: Canadian studies of a global problem, Herpetological Conservation*, St. Louis, Missouri, Society for the Study of Amphibians and Reptiles, **1**: 291-308.

HAY, L. E. & CLARK, M. P., 2003. – Use of statistically and dynamically downscaled atmospheric model output for hydrologic simulations in three mountainous basins in the western United States. *J. Hydrol.*, **282**: 56-75.

HAY, L. E. & MCCABE, G. J., 2002. – Spatial variability in water-balance model performance in the conterminous United States. *J. Am. Water Resourc. Assoc.*, **38**: 847-860.

HAY, L. E., WILBY, R. L. & LEAVESLEY, G. H., 2000. – A comparison of delta change and downscaled GCM scenarios for three mountainous basins in the United States. *J. Am. Water Resourc. Assoc.*, **36**: 387-397.

HAYDEN, B. P., 1998. – Ecosystem feedbacks on climate at the landscape scale. *Phil. Trans. r. Soc. London, Biol. Sci.*, **353**: 5-18.

HAYES, M. P. & JENNINGS, M. R., 1986. – Decline of ranid frog species in western North America: are bullfrogs (*Rana catesbeiana*) responsible? *J. Herp.*, **20**: 490-509.

HAYES, T. B., COLLINS, A., LEE, M., MENDOZA, M., NORIEGA, N., STUART, A. A. & VONK, A., 2002. – Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine and low ecologically relevant doses. *Proc. natl. Acad. Sci. USA*, **99**: 5476-5480.

HELSEL, D. R. & HIRSCH, R. M., 1992. – *Statistical methods in water resources*. Amsterdam, The Netherlands, Elsevier: 1-552.

HOUGHTON, J. T., DING, Y., GRIGGS, D. J., NOGUER, M., VAN DER LINDEN, P., DAI, X., MASKELL, K. & JOHNSON, C. I. (ed.), 2001. – *Climate change 2001: the scientific basis*. Cambridge University, Cambridge, U.K., Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change.

HOULAHAN, J. E., FINDLAY, C. S., SCHMIDT, B. R., MEYER, A. H. & KUZMIN, S. L., 2000. – Quantitative evidence for global amphibian population declines. *Nature*, **404**: 752-755.

JOHNSON, P. T. J. & CHASE, J. M., 2004. – Parasites in the food web: linking amphibian malformations and aquatic eutrophication. *Ecol. Lett.*, **7**: 521-526.

KIESECKER, J. M. & BLAUSTEIN, A. R., 1998. – Effects of introduced bullfrogs and smallmouth bass on microhabitat use, growth, and survival of native Red-legged frogs (*Rana aurora*). *Conserv. Biol.*, **12**: 776-787.

----- 1999. – Pathogen reverses competition between larval amphibians. *Ecology*, **80**: 2442-2448.

KIESTER, A. R., 1971. – Species density of North American amphibians and reptiles. *Syst. Zool.*, **20**: 127-137.

Source : MNHN, Paris

KOONZ, W., 1992. – Amphibians in Manitoba. *In*: C. A. BISHOP & K. E. PETTIT (ed.), *Declines in Canadian amphibian populations: designing a national monitoring strategy*, Canadian Wildlife Service Occasional Paper Nr. 76: 19-22.

KRZYSIK, A. J., 1998. – Amphibians, ecosystems, and landscapes. *In:* M. J. LANNOO (ed.), *Status & conservation of midwestern amphibians*, Iowa City, Iowa, University of Iowa Press: 31-41.

LANNOO, M. J., 1996. – *Okoboji wetlands: a lesson in natural history*. Iowa City, Iowa, University of Iowa Press: 1-156.

LANNOO, M. J., GALLANT, A. L., NANJAPPA, P., BLACKBURN, L. & HENDRICKS, R., 2005. – Ecological and ecoregional analyses of amphibian distributions as an approach to amphibian conservation. *In:* M. J. LANNOO (ed.), *Declining amphibians: a United States' response to the global problem*, Berkeley, California, University of California Press, in press.

LANNOO, M. J., SUTHERLAND, D. R., JONES, P., ROSENBERRY, D., KLAVER, R. W., HOPPE, D. M., JOHNSON, P. T. J., LUNDE, K. B., FACEMIRE, C. & KAPFER, J. M., 2003. – Multiple causes for the malformed frog phenomenon. *In:* G. LINDER, S. KREST, D. SPARLING & E. LITTLE (ed.), *Multiple stressor effects in relation to declining amphibian populations*, ASTM Stock Nr. STP1443: 233-262.

LAURANCE, W. F., MCDONALD, K. R. & SPEARE, R., 1996. – Epidemic disease and the catastrophic decline of Australian rain forest frogs. *Conserv. Biol.*, **10**: 406-413.

LAWLER, S. P., DRITZ, D., STRANGE, T. & HOLYOAK, M., 1999. – Effects of introduced mosquito fish and bullfrogs on the threatened California red-legged frog. *Conserv. Biol.*, **13**: 613-622.

LITTLE, E. E., BRIDGES, C. M., LINDER, G. & BOONE, M., 2003. – Establishing causality in the decline and deformity of amphibians: the amphibian research and monitoring initiative model. *In:* G. LINDER, S. KREST, D. SPARLING & E. LITTLE (ed.), *Multiple stressor effects in relation to declining amphibian populations*, ASTM Stock Nr. STP1443: 263-277.

MONSERUD, R. A. & LEEMANS, R., 1992. – Comparing global vegetation maps with the kappa statistic. *Ecol. Modell.*, **62**: 275-293.

PIELKE, R.A., Sr., MARLAND, G., BETTS, R. A., CHASE, T. N., EASTMAN, J. L., NILES, J. O., NIYOGI, D. D. S. & RUNNING, S. W., 2002. – The influence of land-use change and landscape dynamics on the climate system: relevance to climate-change policy beyond the radiative effect of greenhouse gases. *Phil. Trans. r. Soc. London*, **360**: 1705-1719.

PIELKE, R. A., Sr., WALKO, R. L., STEYAERT, L. T., VIDALE, P. L., LISTON, G. E., LYONS, W. A. & CHASE, T. N., 1999. – The influence of anthropogenic landscape changes on weather in South Florida. *Mon. Weather Rev.*, **127**: 1663-1673.

POUNDS, J. A. & CRUMP, M. C., 1994. – Amphibian declines and climate disturbance: the case of the golden toad and the harlequin frog. *Conserv. Biol.*, **11**: 1307-1322.

POUNDS, J. A., FOGDEN, M. P. L. & CAMPBELL, J. H., 1999. – Biological response to climate change on a tropical mountain. *Nature*, **398**: 611-615.

ROSEN, P. C. & SCHWALBE, C. R., 1995. – Bullfrogs: introduced predators in southwestern wetlands. *In:* E. T. LAROE, G. S. FARRIS, C. E. PUCKETT, P. D. DORAN & M. J. MAC (ed.), *Our living resources: a report to the nation on the distribution, abundance, and health of US plants, animals, and ecosystems*, Washington, DC, US Department of the Interior, National Biological Service: 452-454.

THOMAS, C. D., CAMERON, A., GREEN, R., BAKKENES, M., BEAUMONT, L., COLLINGHAM, Y., ERASMUS, B., FERREIRA DE SIQUEIRA, M., GRAINGER, A., HANNAH, L., HUGHES, L., HUNTLEY, B., VAN JAARSVELD, A., MIDGLEY, G., MILES, L., ORTEGA-HUERTA, M., PETERSON, A., PHILLIPS, O. & WILLIAMS, S., 2004. – Extinction risk from climate change. *Nature*, **427**: 145-148.

WALTER, H., 1973. – *Vegetation of the earth*. New York, New York, Springer-Verlag: 1-237.

YOUNG, B. E., STUART, S. N., CHANSON, J. S., COX, N. A. & BOUCHER, T. M., 2004. – *Disappearing jewels: the status of new world amphibians*, Arlington, Virginia, NatureServe: 1-55.

Corresponding editor: C. Kenneth DODD, Jr.

© ISSCA 2005

Source : MNHN, Paris

New books on frogs and salamanders

Annemarie OHLER

Museum national d'Histoire naturelle
Departement de Systématique et Evolution
USM 602 Taxinomie et Colletion – Reptiles & Amphibiens
25 rue Cuvier, 75005 Paris, France
e-mail: ohler@mnhn.fr

Sergius L. KUZMIN, 1995. – ***The clawed salamanders of Asia. Genus* Onychodactylus. *Biology, distribution, and conservation*.** Magdeburg, Westarp Wissenschaften, Die Neue Brehm Bücherei, Vol. **622**, 1995: 1-112. ISBN 3-89432-425-2.

This short book gives a presentation of various traits of the two species of clawed salamanders. This genus has a large distribution in Asia including Russia, China, Korea and Japon. Therefore most of the studies available have been published in the languages of these four countries and are not easily available for many Western researchers. In this book all this information is now presented in English. The book introduces to the history of the studies of this interesting group of salamanders. The systematics and distribution of the family Hynobiidae are presented in order to explain the taxonomic position of the genus *Onychodactylus*. For both species, data on systematics, morphology, karyology, ontogeny, anatomy, ecology and behaviour are given. The content is thoroughly illustrated by numerous black and white figures and drawings, and two colour plates. This is a complete book about this small genus of salamanders. It will be of interest to all students of biology and evolution of amphibians.

Sally HAINES, 2000. – ***Slithy toves. Illustrated classic herpetological books at the University of Kansas in pictures and conservation***. Society for the Study of Amphibians and Reptiles & The Kenneth Spencer Research Library at the University of Kansas, *Contributions to Herpetology*, **16**: i-vii + 1-184. ISBN 0-916984-53-2.

Sitting late in an autumn afternoon in a old fashioned library and turning the pages of those historical books that spoke for the first time of herps leaves unforgettable souvenirs of a fascinating world of images and stories. Sometimes one is stroke by the fantastic world that cohabits with detailed precise observation, like in the works of Österdam, a student of Linnaeus, where the sketch of a mairmaid gives even more strength to the morphological study of the *Siren*. One is surprised to find all the details of giving birth of a viper in a book dated 1589 at a period where other authors just gave badly outlined figures of quite unidentifiable things. I always was fascinated by the chronological and descriptive details on ontogeny of European frogs that can be found in the book of Roesel of Rosenhof (1758) – a pre-Linnean work according to nomenclature – that is merely known for the high quality of the figures. This author not only was a precise observer of the traits of the species but had also detailed insight in their life history. Beside the strong and intense feeling that fieldwork and observation of animals in their habitat gives to the herpetologist, the discovery of the books and the authors that made up our knowledge is one of the major pleasures of our herpetological work.

Sally HAINES (2000) wrote a nice book that gives a glimpse of this fascinating world of books and she very nicely linked it to the Wonderland of Alice and other stories of Lewis Carroll. It is to invite us to see these mysteries with the open eyes of a child and to be able to make abstraction of theories and disputes that govern our daily work. Sally presents several books, choosing a single plate of each and putting it in its historical frame. In the introduction some explanation on the methodology of printing figures gives technical clues to understand the works. A list of herpetological works containing figures is added and presented both in alphabetic and chronological way.

This book gives a introduction to the fascinating world preserved in the libraries. It allows you to take home a souvenir of those autumn afternoons. It will surely make you return to discover more of these treasures and it will make us attentive to the thoughts and observations of those who preceded us.

© ISSCA 2005

BIBL. DU MUSÉUM PARIS

Source : MNHN, Paris

ALYTES

International Journal of Batrachology
published by ISSCA

EDITORIAL BOARD

Chief Editor: Alain DUBOIS (Laboratoire des Reptiles et Amphibiens, Muséum national d'Histoire naturelle, 25 rue Cuvier, 75005 Paris, France; <adubois@mnhn.fr>).
Deputy Editor: Thierry LODÉ (Laboratoire d'Ecologie animale, Université d'Angers, 2 boulevard Lavoisier, 49045 Angers Cedex, France; <thierry.lode@univ-angers.fr>).
Conservation Editor: Stephen J. RICHARDS (Vertebrates Department, South Australian Museum, North Terrace, Adelaide, S.A. 5000, Australia; <Richards.Steve@saugov.sa.gov.au>).
Editorial Board: Franco ANDREONE (Torino, Italy); Lauren E. BROWN (Normal, USA); Janalee P. CALDWELL (Norman, USA); Ulisses CARAMASCHI (Rio de Janeiro, Brazil); Claude COMBES (Perpignan, France) ; C. Kenneth DODD, Jr. (Gainesville, USA) ; Günter GOLLMANN (Wien, Austria); Heinz GRILLITSCH (Wien, Austria); Tim HALLIDAY (Milton Keynes, United Kingdom); W. Ronald HEYER (Washington, USA); Esteban O. LAVILLA (Tucumán, Argentina); Masafumi MATSUI (Kyoto, Japan); Alain PAGANO (Angers, France); John C. POYNTON (London, England); Miguel VENCES (Konstanz, Germany).
Technical Editorial Team (Paris, France): Alain DUBOIS (texts); Roger BOUR (tables); Annemarie OHLER (figures).
Book Review Editor: Annemarie OHLER (Paris, France).

SHORT GUIDE FOR AUTHORS

(for more detailed *Instructions to Authors*, see *Alytes*, 1997, **14**: 175-200)

Alytes publishes original papers in English, French or Spanish, in any discipline dealing with amphibians. Beside articles and notes reporting results of original research, consideration is given for publication to synthetic review articles, book reviews, comments and replies, and to papers based upon original **high quality illustrations** (such as colour or black and white photographs), showing beautiful or rare species, interesting behaviours, etc.

The title should be followed by the name(s) and address(es) of the author(s). The text should be typewritten or printed double-spaced on one side of the paper. The manuscript should be organized as follows: English abstract, introduction, material and methods, results, discussion, conclusion, French or Spanish abstract, acknowledgements, literature cited, appendix.

Figures and tables should be mentioned in the text as follows: fig. 4 or tab. 4. **Figures should not exceed 16 × 24 cm.** The size of the lettering should ensure its legibility after reduction. The legends of figures and tables should be assembled on a separate sheet. Each figure should be numbered using a pencil.

References in the text are to be written in capital letters (BOURRET, 1942; GRAF & POLLS PELAZ, 1989; INGER et al., 1974). References in the *Literature Cited* section should be presented as follows:

BOURRET, R., 1942. – *Les batraciens de l'Indochine*. Hanoi, Institut Océanographique de l'Indochine: i-x + 1-547, pl. 1-4.

GRAF, J.-D. & POLLS PELAZ, M., 1989. – Evolutionary genetics of the *Rana esculenta* complex. *In*: R. M. DAWLEY & J. P. BOGART (ed.), *Evolution and ecology of unisexual vertebrates*, Albany, The New York State Museum: 289-302.

INGER, R. F., VORIS, H. K. & VORIS, H. H., 1974. – Genetic variation and population ecology of some Southeast Asian frogs of the genera *Bufo* and *Rana*. *Biochem. Genet.*, **12**: 121-145.

Manuscripts should be submitted **in triplicate** either to Alain DUBOIS (address above) if dealing with amphibian morphology, anatomy, systematics, biogeography, evolution, genetics, anomalies or developmental biology, or to Thierry LODÉ (address above) if dealing with amphibian population genetics, ecology, ethology or life history, or to Stephen J. RICHARDS (address above) if dealing with conservation biology, including declining amphibian populations or pathology. Acceptance for publication will be decided by the editors following review by at least two referees.

If possible, after acceptance, a copy of the final manuscript on a floppy disk (3 ½ or 5 ¼) should be sent to the Chief Editor. We welcome the following formats of text processing: (1) preferably, MS Word (1.1 to 6.0, DOS or Windows), WordPerfect (4.1 to 5.1, DOS or Windows) or WordStar (3.3 to 7.0); (2) less preferably, formated DOS (ASCII) or DOS-formated MS Word for the Macintosh (on a 3 ½ high density 1.44 Mo floppy disk only).

Page charges are requested only from authors having institutional support for this purpose. The publication of colour photographs is charged. For each published paper, 25 free reprints are offered by ISSCA to the author(s). Additional reprints may be purchased.

Published with the support of AALRAM
(Association des Amis du Laboratoire des Reptiles et Amphibiens
du Muséum National d'Histoire Naturelle, Paris, France).
Directeur de la Publication: Alain DUBOIS.
Numéro de Commission Paritaire: 64851.

© ISSCA 2005

Source : MNHN, Paris

Alytes, 2005, **22** (3-4) : 65-168

The Amphibian Research and Monitoring Initiative

Proceedings of a Symposium held in Norman, Oklahoma, USA, 2004

Edited by C. Kenneth DODD, Jr.

Contents

P. Stephen CORN, Erin MUTHS, Michael ADAMS & C. Kenneth DODD, Jr.
The United States Geological Survey's Amphibian Research and Monitoring Initiative 65-71

Robin E. JUNG, J. A. ROYLE, John R. SAUER, C. ADDISON, R. D. RAU, J. L. SHIRK & J. C. WHISSEL
Estimation of stream salamander (Plethodontidae, Desmognathinae and Plethodontinae) populations in Shenandoah National Park, Virginia, USA 72-84

P. StephenCORN, B. R. HOSSAK, Erin MUTHS, D. A. PATLA, Charles R. PETERSON & Alisa L. GALLANT
Status of amphibians on the Continental Divide: surveys on a transect from Montana to Colorado, USA 85-94

Wendy H. WENTE, Michael J. ADAMS & C. A. PEARL
Evidence of decline for *Bufo boreas and Rana luteiventris* in and around the northern Great Basin, western USA 95-108

D. Earl GREEN, & Erin MUTHS
Health evaluation of amphibians in and near Rocky Mountain, National Park, (Colorado, USA) 109-129

Christine M. BRIDGES & Edward E. LITTLE
Toxicity to amphibians of environmental extracts from natural waters in National Parks and Fish and Wildlife Refuges 130-145

William BATTAGLIN, Lauren HAY, Greg MC CABE, Priya NANJAPPA & Alisa GALLANT
Climate patterns as predictors of amphibian species richness and indicators of potential stress 146-167

BOOK REVIEWS

Annemarie OHLER
New books on frogs and salamanders 168

Alytes is printed on acid-free paper.
Alytes is indexed in *Biosis, Cambridge Scientific Abstracts, Current Awareness in Bilogical Sciences, Pascal, Referativny Zhurnal and The Zoological Record.*

Imprimerie F. Paillart, Abbeville, France.
Dépôt légal : 2e trimestre 2005.

© ISSCA 2005

Source : MNHN, Paris